SARCOMA DEVELOPMENT OCCURRING DURING THE PROPAGATION OF A HÆMORRHAGIC ADENO - CARCINOMA OF THE MAMMA OF THE MOUSE.

By B. R. G. RUSSELL, M.D.

SARCOMA DEVELOPMENT OCCURRING DURING THE PROPAGATION OF A HÆMORRHAGIC ADENO-CARCINOMA OF THE MAMMA OF THE MOUSE.[1]

By B. R. G. RUSSELL, M.D.

From the Laboratories of the Imperial Cancer Research Fund.
Director, Dr. E. F. Bashford.

(PLATES XVIII.–XXI.).

THE change occurring during the propagation of certain carcinomatous tumours whereby they become converted first into tumours consisting of sarcomatous and carcinomatous elements, which may in turn give place to tumours consisting of purely sarcomatous elements, ranks amongst the more interesting phenomena brought to light by the experimental investigation of cancer.

The number of transplanted carcinomata in which this transformation has been observed has augmented considerably in the last four years (1905–8 [1, 2, 19, 22, 23]). The cases observed (1907) in the laboratory of the Imperial Cancer Research Fund have been fully described by Haaland (1908 [19]), who followed the sarcomatous change in nine different strains of tumour " 37."

With the single exception of a tumour of the rat, all the cases of this transformation have been observed during the artificial propagation of mammary carcinomata of the mouse. It is not proposed here to go at length tino the literature on this subject, as it has been recently critically reviewed by Haaland in the introduction to his paper on " Sarcoma Development." Where necessary, the observations made upon the cases already recorded will be brought under review in the later development of this paper, and the results of the present observations will be used for the critical analysis, so far as possible, of the various theories already enunciated as probable explanations of this peculiar development. The question, whether the tumours produced are true sarcomata, has in our opinion been finally settled by Haaland's (1906 [18], 1908 [19]) observations, and the single dissentient note given by Krompecher (1908 [21]) would not have been sounded had he waited for the full description of the tumour in question.

The question of the origin of these sarcomatous changes in what were previously pure carcinomatous tumours is of extreme importance, bound up as it is so intimately with the general etiology of all neoplastic growths, and a careful analysis of all the cases observed is very desirable.

The sarcomatous change has occurred in another of the propagable mouse carcinomata of the Imperial Cancer Research Fund, in a

tumour with a histological structure different from that of the tumour described by Haaland. We have had the opportunity of following the process and of determining at least some of the factors which play a causal rôle in the production of sarcoma in this instance.

The transplantable carcinomata of the mouse present an infinite variety of growth, so that no two agree in every respect, and it is not to be expected that the development of sarcoma in carcinomatous tumours differing greatly in type will be exactly similar. It is impossible in the present state of our knowledge to formulate a common ætiological factor for all carcinomatous growths, and the same holds good for the sarcomata developing during the growth of carcinomata. The two carcinomatous tumours of this laboratory which have given rise to sarcoma during transplantation do not appear to behave in quite the same manner; yet this difference may be only apparent and the two may have, in regard to sarcoma development, common ætiological factors. The attempt will not be made at present to define the common factors, and the conclusions drawn from a study of the tumour which is the basis of the present communication are not regarded as explaining the behaviour of all carcinomatous tumours which have given and may give rise to sarcoma development.

DESCRIPTION OF PRIMARY TUMOUR "100," AND ITS DESCENDANTS, PREVIOUS TO ONSET OF SARCOMATOUS CHANGE.

The mouse forming the starting-point of the present study was an old female which came under observation on the 9th September 1907, and received the laboratory number $\frac{100}{0}$. She had a tumour in the left groin, oval in shape and about 2 cms. long. This tumour filled up the space between the left thigh and the vulva, reached backwards to the root of the tail, and extended forwards over the lower abdominal region. An attempt was made four days later to extirpate the tumour by an operation conducted under ether anæsthesia. The mouse made a good recovery, the wound healed *per primam*, and four weeks later a recurrent nodule about 1 cm. long had made itself manifest in the scar. This nodule grew rapidly and progressively until the 26th November 1907, when the mouse was killed. The recurrent tumour had then attained a relatively enormous size, and weighed 12 grms. During the growth of this recurrent tumour the total weight of the mouse increased, this increase representing almost exactly the quantity of tumour growth; only during the last week, when the mouse was already showing signs of distress, did an actual decrease in weight take place (*vide* Fig. A).

The material obtained from the first operation showed numerous hæmorrhagic areas, but no distinct necrosis could be noted with the naked eye. A

large slice through the widest diameter of the tumour was preserved in Zenker's solution, whilst the remaining material was used for transplantation into eighty-four mice. Of these mice fifty-seven survived long enough to
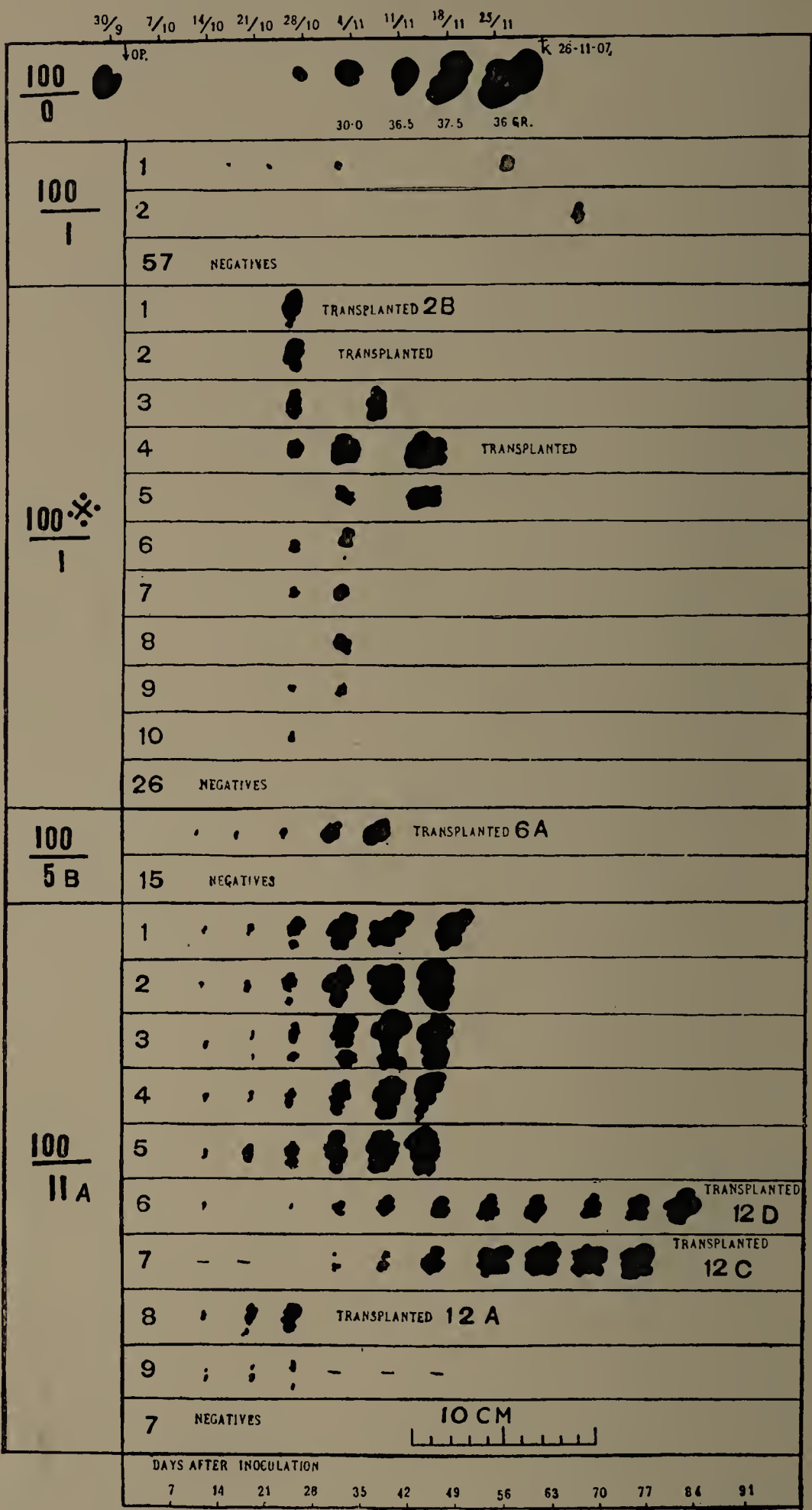

FIG. A.—Chart showing number of tumours obtained in different generations, and the speed of growth compared with recurrence of primary tumour. (× ⅙.)

allow a conclusion to be drawn as to whether growth of the implanted material took place or not. In only two mice, *i.e.* not quite 4 per cent., did any growth take place, and in both, the increase in size of the nodules was very slow, the tumours only attaining a weight of about 0·75 grm. after sixty

days. One of these two daughter tumours was transplanted into twenty mice, and one tumour developed in a mouse which died twenty-one days after inoculation, when the graft had grown to a nodule about 4 mm. in diameter. In addition to the above transplantation into normal mice, grafts of the primary tumour were also inoculated into twenty-eight mice previously inoculated with tumour 27, an adeno-carcinoma, and into twenty-six mice previously grafted with tumour 37, another adeno-carcinoma. In the former series two nodules developed in two mice which were also the bearers of a progressively growing tumour 27. All mice which had been negative to the inoculation of 27 were negative to the re-inoculation with tumour 100. In the second series, only one tumour developed in a mouse carrying a 37 tumour, all the other twenty-five mice remained negative to the inoculation of tumour 100.

The recurrent tumour in the spontaneously affected animal was also transplanted, and gave a series of tumours labelled [1] $\frac{100}{1}$ * (Fig. A). After the animal had been killed, a part of the tumour was removed aseptically for immediate transplantation, and two slices were placed in Zenker's fluid. The rest of the tumour, which had now become firmly adherent to the abdominal and thigh muscles, was left *in situ*, and the whole mouse was preserved in Zenker's fluid, and, after decalcification, cut in serial sections. The lungs, which showed numerous metastases about the size of split peas, were imbedded separately and also cut in serial sections.

Of the sixty-three mice inoculated with material obtained after killing the mouse, only thirty-six survived long enough to judge of the success of the transplantation, and no fewer than ten mice showed progressively growing tumours; *i.e.* 30 per cent. of successes as contrasted with the 4 per cent. of successes obtained in the transplantation of the tumour from the first operation. This increase of the transplantability of the tumour is not to be ascribed to any hypothetical increase of malignancy, the result of surgical interference ; the opposite result has been obtained quite as frequently with various other tumours, as described by Murray (1908 [25]). It is rather to be attributed to cyclical variations in the life of the tumour cells, as described by Bashford, Murray, Cramer, and Bowen (1905, 1906) ([7, 10, 11]).

The microscopical examination of the material from the first operation revealed a type of structure very common amongst the hæmorrhagic mammary tumours (Plate XVIII. Fig. 1). The parenchyma was divided up into larger and smaller alveoli by delicate strands of connective tissue. Some of these alveoli were quite solid throughout, but in most of them, especially towards the centre of the alveoli, the epithelial cells were arranged in the form of small acini, with minute lumina. Over irregular areas a different type of structure was present, the epithelial cells being arranged in narrow, parallel columns lying in a delicate œdematous tissue, whilst scattered irregularly between these columns were small groups of acini. The stroma throughout the whole tumour was very delicate, and consisted of slender spindle-shaped connective-tissue cells lying upon fine bundles of collagen fibres. The stroma contained numerous large dilated blood vessels, whose wall consisted only of a single delicate layer of

---

[1] The numerator 100 refers to the laboratory number of the tumour, the star denotes that the parent material was obtained from a recurrence of the primary growth. The denominator refers to the generation attained through transplantation, to which is later added a letter of the alphabet signifying the series in that generation.

endothelial cells.   The distribution of these large blood sinuses throughout the tumour was very irregular, and in many places the vessel wall had given way, allowing the blood to pass into the parenchyma of the tumour amongst the epithelial cells.   The tumour presented the characters typical of hæmorrhagic mammary carcinomata of the mouse, as described in the Second Scientific Report of the Imperial Cancer Research Fund (1905), and later by Apolant (1906 [3]) and Gierke (1908 [15, 16]).   There were no signs of necrosis in any of the tumour constituents; all of the epithelial cells appeared quite healthy, and many were in a state of division.   The tumour had already invaded and in part destroyed the adjacent muscular tissue; the type of growth of the tumour in this muscular tissue was markedly acinous. The two daughter tumours of this material which were also examined presented in every respect a similar microscopical structure.

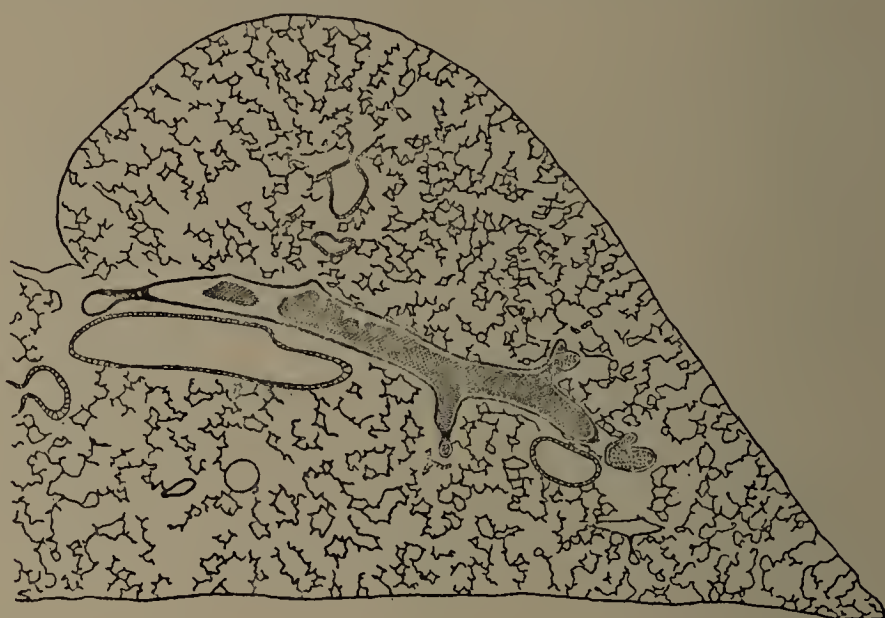The recurrent tumour in the primarily affected mouse retained

Fig. B.—Primary tumour, 100.   Carcinomatous embolism
of pulmonary artery.   ($\times \frac{17}{1}$.)

essentially the structure of the tumour just described, excepting in two particulars.   There were present in the centre of the tumour, and also in isolated areas throughout the periphery, large and small areas of necrosis such as are always found in rapidly growing tumours, where the blood supply is unable to keep up the pace of development set by the tumour.   The large dilated blood vessels were not so numerous as in the material from the first operation, but hæmorrhage was again present, occurring especially as the result of the involvement of a blood vessel in a necrotic area.   The stroma was very delicate, only in one or two small areas adjacent to the necrotic foci had it become slightly more abundant and more cellular.   The pulmonary metastases, as seen in stained sections, varied in size from small points up to circular nodules about 3 mm. in diameter.   Fig. B, a low-power sketch of a lobule of the lung, shows a large branch of the pulmonary artery which is injected with a solid mass of carcinomatous cells, in a manner similar to that described by Borrel (1903 [13]),

Haaland (1905 [17]), Bashford, Murray, and Cramer (1905 [7. 9]), for mouse tumours. This intravascular growth, excepting for a delicate layer of endothelial cells over the surface, was quite devoid of any supporting tissue, and accordingly showed no arrangement of the parenchyma into acini or alveoli. An earlier stage of this condition is shown in Plate XVIII. Fig. 2, where one end of a small artery is occupied by an aggregation of carcinoma cells. The perivascular space of this vessel shows secondary changes of an inflammatory character. Further on in the series of sections this intravascular growth breaks through the vessel wall, proliferates first in the perivascular space, and later through the lung tissue generally. In all the large nodules the growth was of a very pronounced acinous character, the blood vessels were only of a medium size, and the stroma was very delicate (Plate XVIII. Fig. 3). The elastic tissue of the lung was present in abundance at the periphery of the metastases, around which it was arranged concentrically, the result of the rapid growth of the tumour cells.

Of the ten tumours obtained in the series 100*/1 two were transplanted and examined; the first tumour, 27 days old, gave series 100/2B, the second tumour, 43 days old, gave series 100/2C.
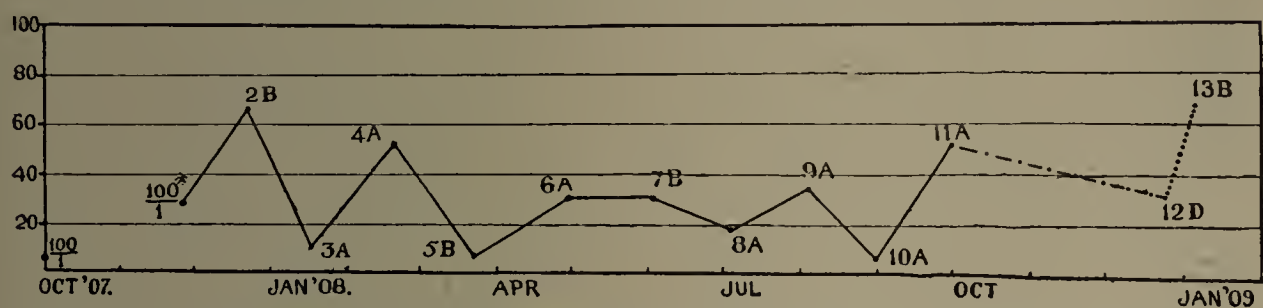

FIG. C.—Percentage curve of propagation of tumour 100, showing rise and fall in transplantability in different generations, and the rise when carcinoma was replaced by sarcoma. ——— Carcinoma, — . — . — . — Carcino-sarcoma, ... Sarcoma.

The percentage of success from these two inoculations, as reckoned by dividing the total number of mice remaining alive after three weeks by the number of progressively growing tumours, was 66 per cent. and 4 per cent. respectively. It is from the former series, 100/2B, that the main stem of this tumour has been propagated, and whilst occasionally other side stems have been carried on for two or three generations, none of them has been continuously propagated, and series 9A, from which all our present material with its numerous sarcomatous descendants is derived, is the direct lineal descendant, through seven generations, of series 2B. Reference to Fig. C, which is a percentage curve of the propagation of this tumour, shows at a glance the line of propagation. The ordinates represent percentages of successes, whilst the abscissæ represent the dates on which transplantation was carried out.[1] When the experiments are so arranged it will be seen that the transplantability of the tumour is a very inconstant factor. Thus the rise on passing from the first to the second generation is followed by a sudden drop on passing into the third, which, in turn, is followed by a second rise and a second drop. These fluctuations have been analysed in a

---

[1] The interpretation and the method of constructing such charts is fully described by Bashford, Murray, and Bowen, "The Experimental Analysis of the Growth of Cancer," *Proc. Roy. Soc. London*, 1906, vol. lxxviii. B.

paper by Bashford, Murray, and Bowen, and, as already noted, are referred by them to cyclical changes in the cancer cell. In this tumour there is no gradual increase in transplantability from generation to generation, comparable to what is termed an increase in virulence as the consequence of repeated *passage* of an organism through suitable animals ; a reference to the percentage curve shows that the carcinoma gave a higher success at the second passage than in the succeeding generations. It is true that recently even 95 per cent. of successes have been obtained, but this has only occurred after a great many series had been laid down for the purpose of following the sarcoma development, and against this high percentage of successes have to be reckoned those parallel series, of equally recent origin, where no tumours have developed at all, or perhaps only one tumour in twenty mice inoculated. Such enormous variations in success as these cannot be attributed to experimental error. The inoculability varies enormously, even where precautions are taken to ensure similarity of age and weight of mice, age and dose of tumour inoculated, where the method and the site of inoculation are the same, and where the personal factor is allowed for by having the inoculations carried out by the same experimenter.

Another factor closely allied to, but not identical with, the former is the rate of growth of the tumours inoculated. To obtain the necessary data for this, it is usual to have a silhouette drawn of the sizes attained by the tumours in the several mice. This is done ten or fourteen days after the inoculation, and the observation is repeated once a week. Fig. A gives such a chart for several series of tumour 100 in various generations. The tumours have all been drawn to the same scale, and they are aligned so that the dates of inoculation fall on the same perpendicular line. The day on which·the operation was performed on 100/0 has also been made to align with the dates on which subsequent sub-graftings were made, so that a comparison is possible of the rate of growth of the recurrent primary tumour with the implanted tumours of the subsequent generations. When the recurrent tumour is compared with the daughter tumours of the primary 100/1 and its own daughter tumours 100*/1, it will be seen that although twenty-eight days elapsed before the recurrent growth was manifest, yet, once it showed itself, growth was more rapid than in any of the daughter tumours. It even bears favourable comparison with the rate of growth exhibited by series A of the eleventh generation, also shown in Fig. A, which gave a fairly high percentage of successes, namely, 50 per cent. Of the hundreds of carcinomatous tumours obtained through transplantation of this strain, not one has exceeded in rapidity of growth the recurrence of the primary tumour. Series 5B is also given in this figure to illustrate the somewhat slower rate of growth usually present when the transplantability of the tumour is also low.

Of the two methods of propagating tumours employed in the laboratory, by the needle and by the syringe, tumour 100 gives very much better results with the former. When inoculations are done with the needle, the weight of the graft inoculated varies from 0·01 to 0·02 grm., but where the syringe method is used much larger doses can be given, even up to 0·25 grm. Had tumour 100 grown well with the syringe method, it is conceivable that very much larger tumours would have been obtained within a given period, from the mere fact that the initial number of cells capable of multiplying would have been greater, but even then there would have been no justification for assuming that the tumour cells had undergone any increase in their rate of proliferation, *i.e.* in their rate of assimilation of food stuffs. As has so frequently been urged from this laboratory, there would only be grounds for assuming an increase in the adaptation of the cells, and that an increasing number survived transplantation.

As already stated, of the daughter tumours of the recurrent growth, two tumours, No. 1 and No. 2 of Fig. A, 100/1, were transplanted, one giving series

2B and the other series 2c. The former tumour reproduced the characters of the primary growth, except that the large dilated blood vessels were absent, but in the latter tumour, giving 2c, the hæmorrhagic nature of the tumour was again evident. Up to the ninth generation fifteen other tumours were transplanted and a histological examination made of each. As there was present no special change in the minute anatomy, a detailed account of their histology can be omitted. Sometimes the alveolar arrangement of the parenchyma would predominate over the acinous, and *vice versa*. The stroma, fairly abundant and rather cellular in one tumour, might be replaced in the next generation by a stroma reduced to a minimum. The large blood sinuses were also developed to a very irregular degree in succeeding generations. In none of these tumours was there present any change in the stroma which gave any suspicion of ultimate sarcoma development.

Thus far we have studied three characters of this tumour, first the transplantability, secondly the rate of growth, and thirdly the morphology. Judged by these three characters, the cells of the propagated tumours do not differ materially from those of the primary growth. There has been observed no change produced by the continuous cultivation of the tumour cells in other mice which could be ascribed to an increase in rate of proliferation or to a biological alteration in the tumour cells.

DEVELOPMENT OF SARCOMA IN DIFFERENT STRAINS OF TUMOUR 100, PROPAGATED IN PARALLEL SERIES.

From series A of the ninth generation has been derived all the material with which the later transplantations have been carried out.
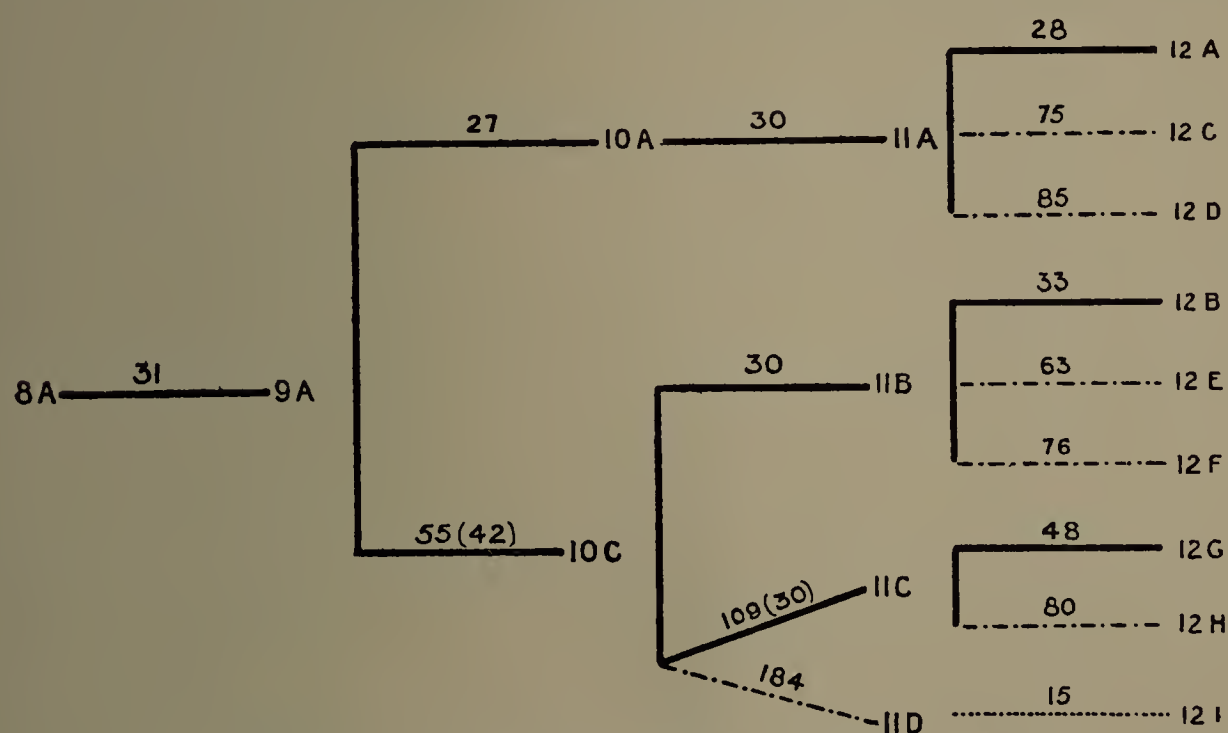

Fig D CARCINOMA ———— MIXED TUMOUR ·—·—· SARCOMA ············
Genealogical tree of tumour 100 for the 8-12th generations: the numbers above the lines give the ages in days of the tumours.

Fig. D gives, in the form of a genealogical tree, the later transplantations up to the twelfth generation. The number above the lines in the tree refers to the age of the tumour at the time it was used for

inoculation, and where this number is followed by another number in brackets, the latter indicates the number of days during which growth had been evident. It is not unusual to observe in this tumour that the inoculated graft remains quiescent over a long period and then commences to grow, and, as will be seen later, it is of importance to know exactly the duration of time during which growth has been going on.

From series 9A a tumour was transplanted giving series 10A, and from the latter one tumour was transplanted giving 11A. Both of these tumours were pure carcinomata.

From series 11A three tumours were examined and transplanted. One tumour, which gave series 12A, was still a pure carcinoma, but the second tumour, which gave series 12C, presented unusual changes in its stroma suggestive of those described by Bashford, Murray, and Cramer (1905 [7, 8]) as occurring during the spontaneous absorption of a tumour, and very like those described by Haaland as typical of the
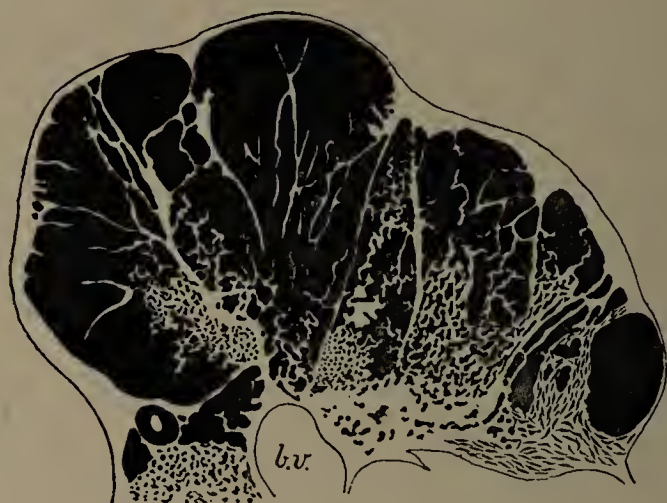

FIG. E.—100/11A–12C. Tumour, 75 days old, showing sarcomatous transformation of stroma at one side. (× $\frac{6}{1}$.)

onset of sarcoma development. For the greater part of the tumour the parenchyma and stroma retained their usual features and relative proportions; but, over a sharply defined limited area close to the periphery of the tumour, the stroma had increased greatly in bulk and become very cellular, as is shown schematically in Fig. E. The mesoblastic tissue of this area was composed of spindle-shaped cells arranged in columns, which interlaced and crossed each other in the manner characteristic of the sarcomata. The nuclei of these cells were large, oval in shape, and fairly rich in chromatin. Judging by the number of mitotic figures, this tissue was growing very actively. The carcinomatous alveoli for the most part were sharply demarcated off from this tissue, although in one or two places the spindle-shaped cells were beginning to penetrate into the alveoli in the neighbourhood and obscure the otherwise sharp line between the epithelial and mesoblastic cells. Some small islands of epithelial cells were lying isolated in the centre of this sarcomatous tissue.

A third tumour of this series (11A–12D) was examined in the

manner described by Haaland and then transplanted. Here it was found that the change had gone much further. Nearly the whole of one end of the tumour was pure spindle-celled sarcoma, whilst the other end, excepting one or two small bands of sarcoma, was carcinomatous.

From series 9A another tumour, æt. 55 days, had also been transplanted, giving series 10c. Although fifty-five days had elapsed since the introduction of the graft which gave rise to this tumour, macroscopical evidence of growth could only be detected during the latter forty-two days. No evidence of a sarcomatous transformation of the stroma was detectable in this tumour. From 10c a tumour was transplanted giving 11B; this tumour was also a pure carcinoma. Three tumours of 11B were examined and transplanted,—one, a pure carcinoma æt. 33 days, gave 12B, and the two other tumours which already exhibited the sarcomatous changes gave 12E and 12F. The ages of the latter tumours were 63 and 76 days respectively.

Thus far there had been examined four tumours showing sarcoma development, and varying in age from 63 to 85 days. None of their parent tumours, all of which had remained pure carcinomata, had ever grown in any single mouse for such a long time. It appeared, therefore, as if long residence in one animal played a part in producing this peculiar change in the stroma of the tumours. To prove if this were the case or not, the attempt was made to retain the tumours in one animal for a long period, either by choosing somewhat larger mice, or by partially excising the tumour when it had attained to a large size and was threatening to destroy the mouse through ulceration of the overlying skin.

A large tumour of 10c, number 9, was chosen for operation. At the time of the operation 109 days had elapsed since the date of inoculation, but no palpable growth had been formed until thirty days before the operation. A frozen section of this tumour was examined about three hours after the operation, and found to be pure carcinoma. The rest of the tumour which had been kept in the ice chest was then transplanted into twenty mice giving series 11c. Three weeks later a second operation was performed on the recurrent tumour, and a small tongue of sarcomatous looking tissue was found at one side of the tumour, and a second small area in the centre as seen in Fig. F. Material from this operation was not transplanted and is not represented in Fig. D. Seven weeks later the mouse was killed and the distribution of the sarcomatous and carcinomatous areas in the second recurrence is given in Fig. G. Material from the autopsy was transplanted into series 11D, and gave in the daughter tumours a pure sarcoma with irregular shaped cells, but generally spindle-shaped in contour. A third tumour of series 10c presented a very peculiar type of growth. The tumour grew progressively for the first five weeks after inoculation, and then diminished to a small nodule, which thereafter varied in size for a few months and finally began to grow rapidly. The resulting tumour was excised 202 days after inoculation, and was found to be a pure spindle-celled sarcoma.

Two daughter tumours developed in series 11c, and both were examined previous to transplantation. One at forty-eight days remained pure carcinoma, and was transplanted, giving series 12G. The second, at eighty days, showed

towards its centre, and, extending out to the periphery at one side, an area over which large masses of spindle-celled sarcomatous tissue were intercalated between the carcinomatous alveoli. It was also transplanted and gave series 12H, as shown in Fig. D.

The behaviour of tumour 100 has thus been followed to the twelfth sub-transplantation or generation, and to elucidate the further propagation the genealogical tree will be broken into four main branches, which can be discussed separately.

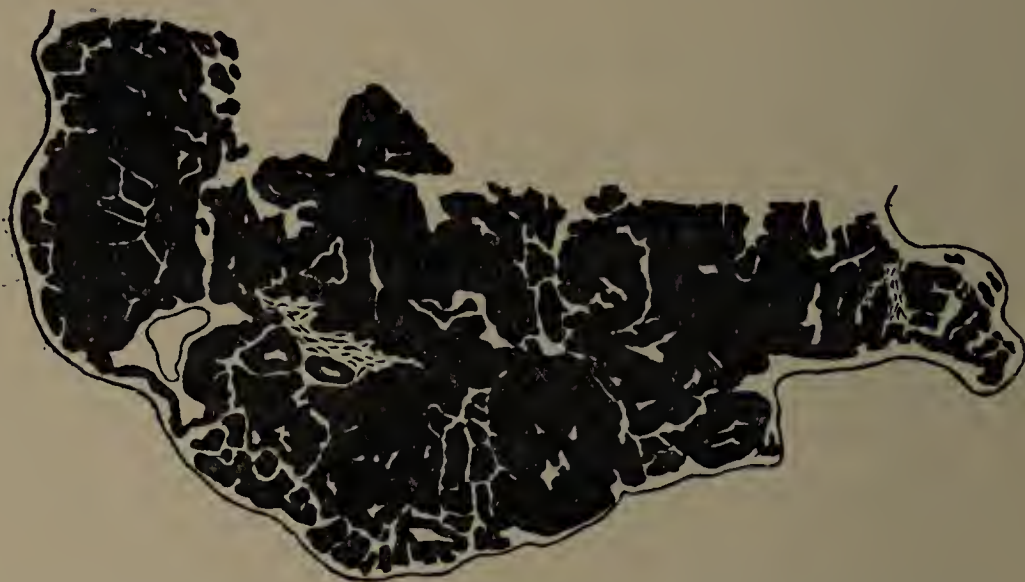Fig. H gives the branch springing from series 12D which came

FIG. F.—100/10C, No. 9. Recurrent tumour from first operation ; 129 days after inoculation. Shows two small areas of sarcomatous change in stroma. (× $\frac{4}{1}$.)
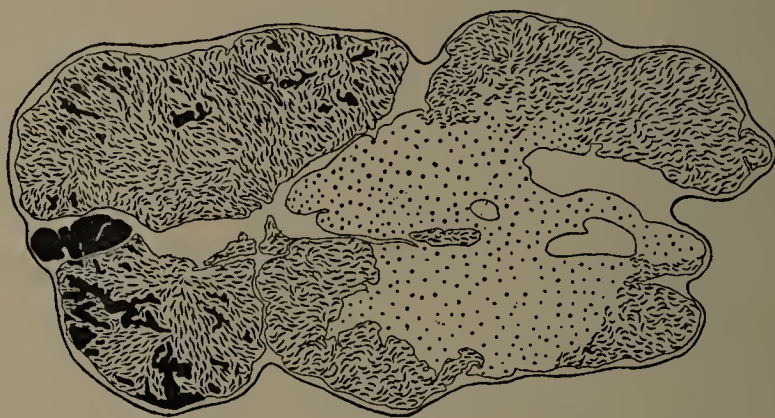

FIG. G.—100/10C–11D. Recurrent tumour from second operation ; 184 days after inoculation. Shows excess of sarcoma, in part necrotic. (× $\frac{3}{1}$.)

from 11A, and was the second case noted as giving sarcoma development.

Small grafts of the sarcomatous tissue of this tumour were specially picked out and inoculated into twelve mice, and then several mice were killed at twenty-four hours, forty-eight hours, etc. Microscopical examination of these "early stages" showed that the spindle cells continued to live in the new host, and were already dividing actively after twenty-four hours. This is exactly what happens when spontaneous sarcomata are transplanted. Of thirty-eight other mice inoculated with material, selected as being carcinomatous, sarcomatous, and mixed, thirteen developed tumours, and of these thirteen tumours six were transplanted and examined. The first daughter tumour transplanted (12D–

13A) was a mixed tumour, with the sarcomatous elements preponderating. The mixture of the two elements was very intimate, the sarcomatous tissue being closely applied to the epithelial tissue and forming a " stroma " for the epithelial cells which were present, mostly in the form of narrow bands with irregular thickenings, radiating through the tumour. In the next generation this mixed tumour freed itself from the carcinomatous component, and has subsequently been propagated as a pure, spindle-celled sarcoma through seven generations (Plate XXI. Fig. 8).
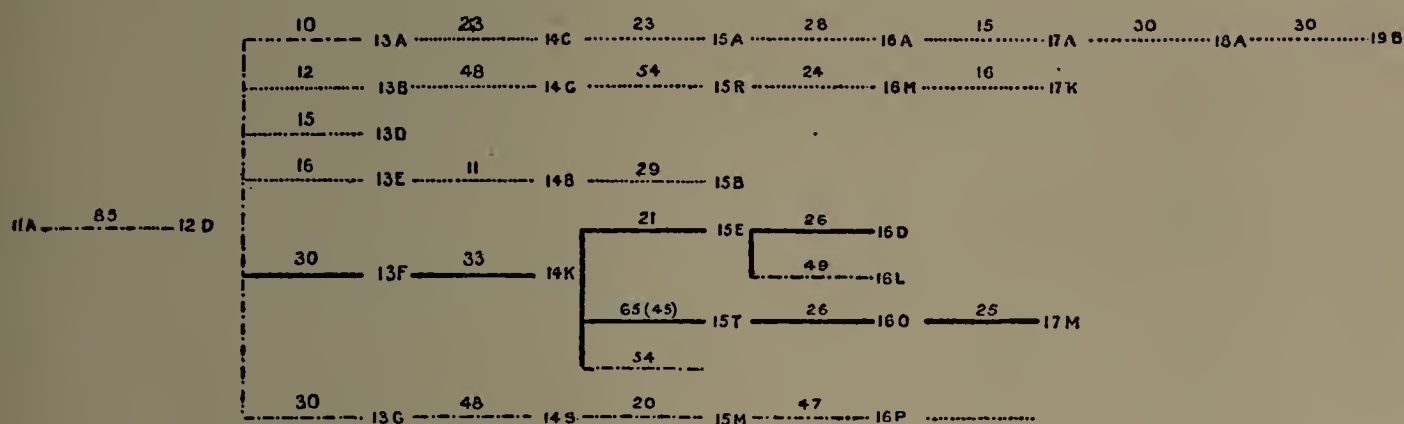

Fig. H. CARINOMA ———— MIXED TUMOUR ·—·—·—·SARCOMA ···············
Genealogical tree of tumours descended from series 12 D: the
numbers above the lines give the ages in days of the tumours.

Three other tumours of 12D which were transplanted were already pure spindle-celled sarcomata. A fifth tumour (12D–13F) was a pure carcinoma, and a sixth (12D–13G) was mixed.

The explanation of the variations in histology of these daughter tumours lies in the method of inoculation and in the distribution of the sarcomatous tissue throughout the parent tumour. It was previously remarked that tumour 11A–12D was nearly all sarcoma at one end and pure carcinoma for the greater part of the rest of the tumour. This distribution was ascertained from a frozen section previous to the transplantation of the tumour, according to the method devised by Haaland, and, as described above, the attempt was made
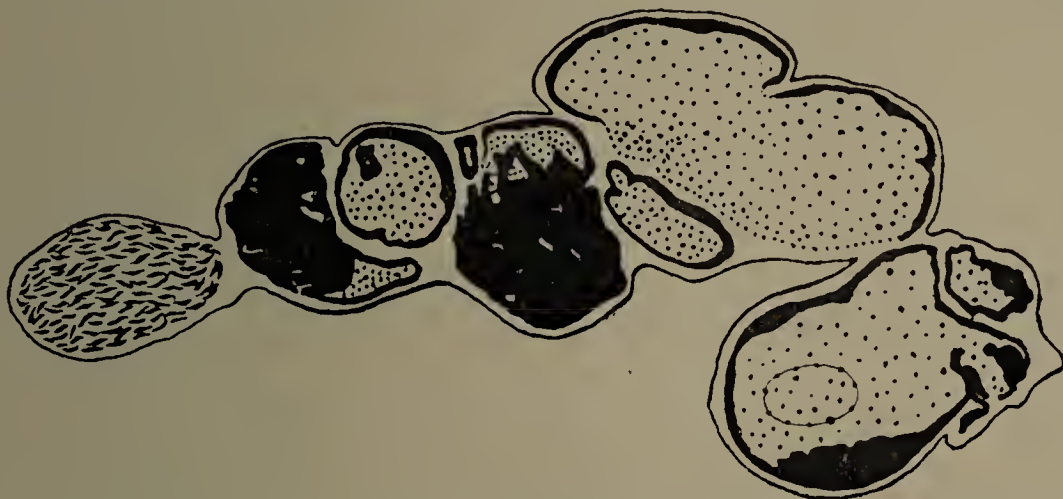

FIG. I.—100/14s, No. 12. Tumour, 46 days old, showing cystic degeneration of carcinoma, and segregation of carcinoma and sarcoma after inoculation of a mixed emulsion of these two tumours. (× $\frac{4}{1}$.)

by picking out small fragments (0·01–0·02 gr.) to segregate the various components of the tumour. This explains the pure carcinomatous character retained by the tumour 12D–13F. Another form of segregation has been obtained by Apolant (1908 [5]) when attempting to produce mixed tumours artificially, by inoculating a mixture of pure carcinoma and pure sarcoma. He obtained in some cases genuine carcinoma-sarcomatodes, whilst other sister tumours were pure sarcomata or pure carcinomata. Fig. I shows this segregation occurring in one animal for tumour " 100."

None of the six daughter tumours in 13F attained to an age of 60 days, and all remained pure carcinoma. Only one of them was transplanted, giving

series 14K. In series 14K four tumours were killed and one transplanted twenty-one days after inoculation. In none of them could any trace of sarcomatous tissue be detected. Another tumour of this series which had grown progressively from the date of inoculation, and had attained, after fifty-four days, a weight of 11 grs., was killed and examined. In this tumour indubitable evidence of a sarcomatous transformation of the stroma was present. Another very old tumour of this series also showed a similar change. By choosing young tumours for propagation, this strain has been carried three generations further, still as pure carcinoma. One tumour, 15E–16L, descended from this strain showed the development of sarcoma forty-nine days after inoculation.

The sixth tumour propagated from 12D, giving 13G, was a mixed tumour, and an attempt was made to see if it were possible to maintain this mixed character in the succeeding generations. Before the tumour was transplanted it was cut up into an emulsion to ensure thorough admixture of the carcinoma and sarcoma cells, and then inoculated by means of a syringe and needle. By using this method of propagation the tumour was kept as a mixed tumour through four generations, but eventually the sarcomatous elements replaced the carcinomatous.

The next main branch to be considered came from a tumour of 11B, giving 12E (*vide* Fig. J). The tumour, æt. 63 days, and

FIG J. CARCINOMA ——— MIXED TUMOUR —·—·— SARCOMA —·····—
Genealogical tree of tumours, descended from series 12E: the numbers above the lines give the ages in days of the tumours.

weighing 10 grs., showed, towards the centre, two large patches of sarcoma quite distinct from each other in the sections examined. Over large areas of the tumour the stroma appeared to be quite normal (cf. Fig. F).

Here, again, segregation of the components was attempted by using the needle method of inoculation, and was successful. Two mice were killed, and the young daughter tumours were pure carcinomata. Both were transplanted, giving 13I and 13L. An examination of the site of inoculation after twenty-four hours, etc., was also carried out to determine the fate of the implanted stroma. No evidence could be seen of any further growth on the part of the introduced stroma cells; the carcinomatous cells alone continued to divide, and were later revascularised by a proliferation of the mesoblastic tissue of the new host. A third carcinoma strain, 13K, was obtained by operating upon a young tumour, and here again the stroma behaved similarly upon transplantation. This same tumour has now been operated on four times (each time a small fragment has been purposely left behind), and when the mouse was killed, thirty days after the fourth operation, the large recurrent growth was still a pure carcinoma.

Two other tumours of series 12E were operated on fifty-three days after

inoculation, and one was transplanted, giving 13o. Both had already a mixed character, and in the recurrent growth the same type was retained In one the sarcoma cells had arranged themselves in a ring round the carcinomatous alveoli, somewhat like the halos seen in our tumour 37, described by Haaland. The resemblance was further enhanced through the presence of numerous multinucleated giant cells in the sarcomatous tissue. This, however, is the only instance in which this arrangement has been observed in tumour 100, and is not a characteristic of its mixed-tumour stage. The second tumour, which was operated on three times and had become mixed, produced a metastatic deposit about the size of a pea in the upper lobe of the left lung. This secondary deposit was also a mixed tumour.

Fig. J gives the result of the further propagation of this branch. It will be seen that three sub-series have maintained their pure carcinomatous character throughout six generations. The tumours which have been utilised for transplantation have designedly been as young as was compatible with the provision of the requisite quantity of material. Further, it has been customary to preserve two or three young tumours in each series, at or about the thirtieth day, in order to possess an efficient control over the histology of the tumour used for transplantation. The remaining tumours in each series have then been allowed to grow until impending ulceration required the destruction of the animal. It has not been possible to obtain in every series tumours which had even approximated to 50 or 60 days' growth in any one animal. Very old tumours have, however, been obtained in series 14H, 15I, and 16H, and all have shown the sarcomatous change in the stroma. Several other mixed tumours derived from pure carcinomata are shown more especially in the tumours of the fourteenth passage, e.g. 13I to 14U, 13I to 14Z, 13L to 14$_2$A, and a tumour of 13K. The four were all very old tumours, and could be used as evidence in favour of the origin of sarcoma in old carcinomatous tumours, more especially as the young sister tumours were in each case pure carcinomata. It is not intended, however, to use these four as a support to this view, and the following example will explain the reason. The graft from which tumour 12E to 13I developed came from a mixed tumour of series 12E, and might therefore have contained sarcoma cells. Actually no sarcoma cells could be seen in either of the two sections examined, nor could their presence be detected in the material used for the examination of the behaviour of the tumour 24 hours, 48 hours, etc., after transplantation. The portion of this tumour which provided the grafts from which some of the daughter tumours in 13I developed might possibly have contained sarcoma cells not present in the portion examined histologically, and it is in the daughter tumours that we must look for their development. Tumour 13I to 14H is one of several young daughter tumours which all failed to show sarcoma ; it is only when old tumours such as 13I to 14U, and 13I to 14Z are examined, that a sarcomatous element is present in the tumours. Thus all the facts speak against the transmission through the graft 12E to 13I of certain sarcoma cells, which remained hidden and were only detected in the old daughter tumours 13I to 14U and 13I to 14Z ; but although improbable, the possibility of such a transmission exists for this specific instance and the segregation of pure carcinoma in 12E to 13I may in this sense have been taken to be apparent and not real. The criticism which has thus been made against the origin of the sarcoma elements in these four tumours must not be made applicable to the later instances of sarcoma development in this branch, as, for example, in a 63-days' old tumour of 16H. The ascendants of this tumour were pure carcinomata in each of the four preceding generations, and it is quite beyond the range of possibility that sarcoma cells could have been transmitted through these without revealing their presence.

The third main branch of the tree sprang from a 76 day old

tumour of series 12F (*vide* Fig. K).   The tumour, which weighed 7·5
grms., showed a very extensive distribution of the sarcomatous tissue
(*vide* Fig. L).   A high power view of a small part of this tumour
is given on Plate XIX. Fig. 5, and shows the character of this
sarcomatous tissue, as also the intimate relations between it and the
carcinomatous alveoli.   In this mixed-tumour stage there is often
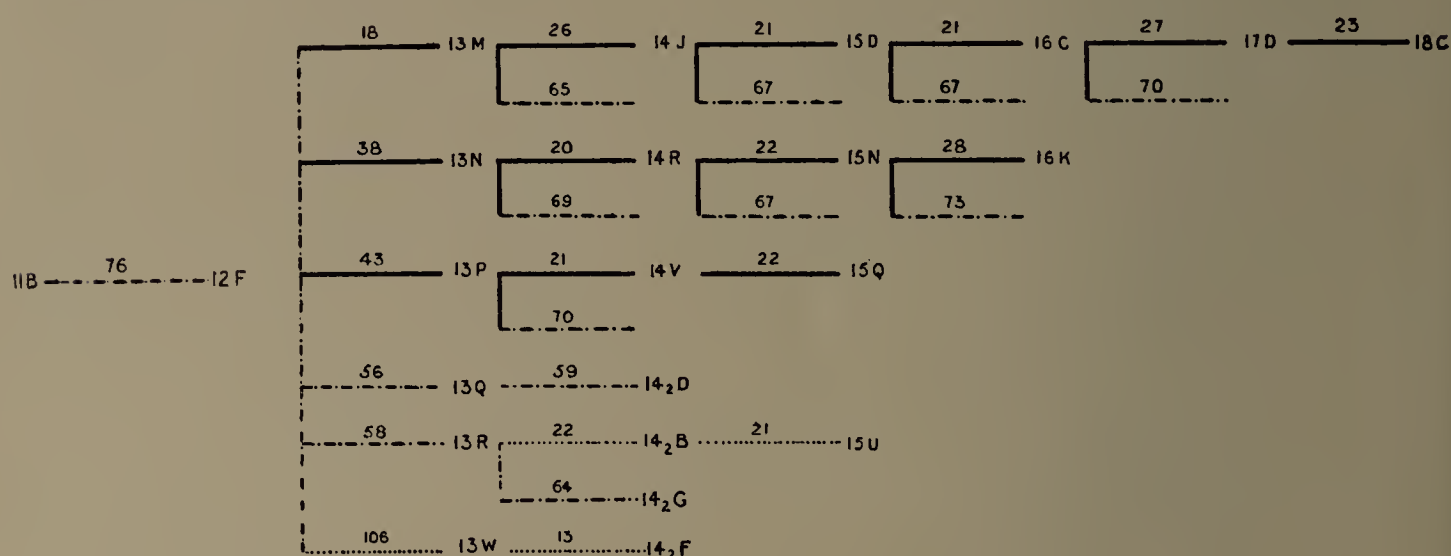greater diversity in shape and size of the sarcoma cells than is met

Fig. K   CARCINOMA ———MIXED TUMOUR—-—SARCOMA ·········
Genealogical tree of tumours descended from series 12 F: the
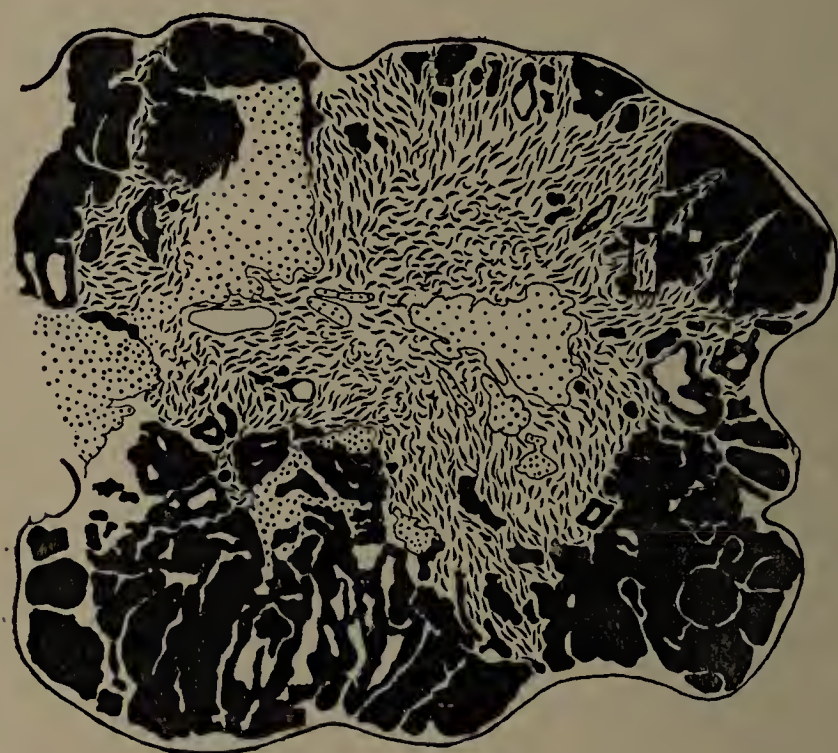numbers above the lines give the ages in days of the tumours.

Fig. L.—100/11B–12F.   Tumour, 76 days old, showing periphery
mostly pure carcinoma, with central area mostly pure sarcoma.
($\times \frac{4}{1}$.)

with in the later stage when the tumour has become pure sarcoma
(cf. Plate XXI. Fig. 8).   The stroma over a limited area of this tumour
had, however, still the characters of normal stroma.   This area was
located by examining a frozen section of the tumour before transplanta-
tion, and again the attempt was made to segregate this area and to
inoculate small grafts containing only the carcinomatous elements.
As seen on Fig. K, three carcinomatous tumours were obtained, which

by rapid passage have retained the carcinomatous structure for six, four, and three generations respectively. "Early stages" of the mixed material of the tumour were also prepared, and in these the carcinoma and sarcoma cells were seen growing side by side twenty hours after inoculation, as shown in Plate XX. Fig. 6.

Two older daughter tumours, of 56 and 58 days growth respectively, were mixed tumours and were transplanted, giving series 13Q and 13R. The sub-transplant of one of them, 13R to 14₂B, was a pure sarcoma. The cells of this sarcoma were more polymorphous, round, and oval-shaped cells of various sizes mingled irregularly, and towards the centre of the tumour, bordering on a necrotic area, numerous multinuclear giant cells had developed. The sixth tumour transplanted from series 12F was a very old tumour which had been growing for 106 days. Its carcinomatous part had been completely replaced by a sarcomatous tissue composed of oval or fusiform cells, and the daughter tumours after ten days' growth had attained to a size seen in the carcinomatous tumours of 100 only after three or four weeks.
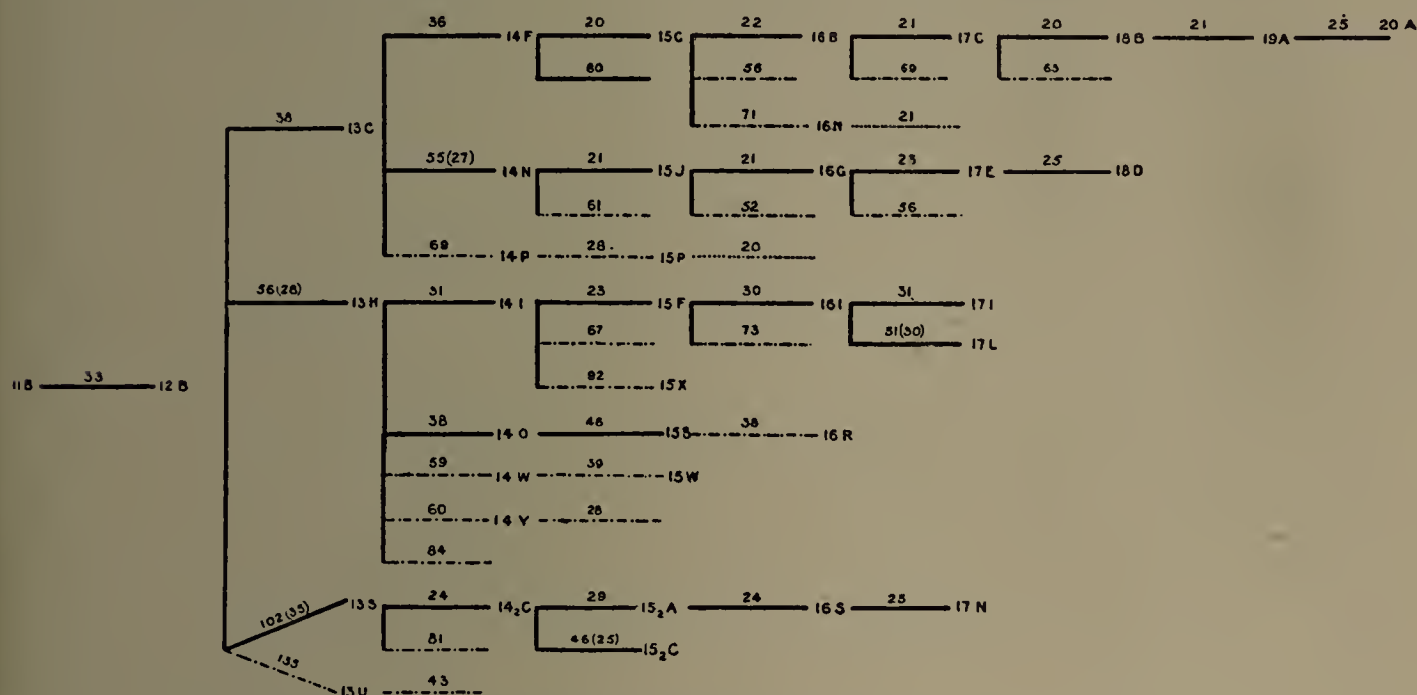

Fig M. CARCINOMA ——— MIXED TUMOUR - - - - SARCOMA ·········
Genealogical tree of tumours descended from series 12 B: the numbers above the lines refer to the ages of the tumours in days.

The further propagation of the series 12F to 13M gave rise to a strain which exemplifies in the most characteristic manner the behaviour of tumour 100 as regards sarcoma development. On referring to the top line of Fig. it will be seen that the young tumours have, for six generations, remained pure carcinoma, and yet in four of these generations the old tumours have become mixed with sarcoma at the sixty-fifth, sixty-seventh, sixty-seventh, and seventieth day respectively. This is a paradigm of tumour 100, and where other series have not shown this so clearly it is merely due to the technical difficulties attached to the maintenance of growth over such a long period in one animal. From series 12F, twenty tumours in all were examined, but they merely confirm more strongly the results of the examination of the six which were also transplanted.

The consideration of the fourth main branch, series 12B and its offshoots, has been reserved to the last. The tumour giving series 12B shares with the tumour giving series 10C the distinction that all its ascendant tumours were pure carcinomata. The observations made upon 12B and its descendants carry more conviction than those made upon 12D, 12E, and 12F.

Fig. M gives the further propagations from series 12B. The parent tumour of series 12B had rather more stroma than usual, and special attention was paid in the daughter tumours to the behaviour of this stroma on transplantation. The first tumour of series 12B was transplanted thirty-eight days after inoculation, and gave 13c; histologically, it repeated the structure of the parent tumour. "Early stages" of this material were also examined. The parenchyma of the tumour went on growing in the new host, but no evidence of growth of the introduced stroma could be seen (cf. Plate XX. Fig. 7). A new stroma was formed from the third to the fourth day by the proliferative activities of the surrounding tissues of the new host. This is the course usually pursued by a pure carcinoma on introduction into a fresh host, as described by Bashford, Murray, and Cramer in 1905. From series 13c a 36-day old carcinomatous tumour was transplanted giving 14F. This line has now attained to the twentieth generation, and is still a pure carcinoma, whenever young tumours are selected for propagation. As shown on Fig. M, the old tumours of those series have invariably shown the presence of sarcoma after long sojourn in one animal. An exception to this occurred in series 14F, in which an old tumour, after growing eighty days in one animal, still retained a purely carcinomatous type of growth. It is, however, of interest to note that during the last ten days of its residence in this animal the tumour had decreased in size, which is sure evidence of a retrogressive change in the epithelial constituents.

A second tumour of series 13c was operated upon fifty-five days after the date of inoculation, but only twenty-seven days after the onset of macroscopic growth. The material obtained was pure carcinoma, and was transplanted, giving series 14N. By selecting young tumours in each generation, this strain has been propagated up to the eighteenth generation as a pure carcinoma, while in the different series those tumours which have been allowed to remain for a long period, fifty to sixty days in one animal, have become carcino-sarcoma. The tumour of 13c which gave 14N recurred, and was operated upon a second time, thirty-five days after the first operation. The material differed from that obtained at the first operation, in showing distinct sarcomatous changes in the stroma. Twenty-four days after the second operation, the mouse was killed on account of its having contracted enteritis. The post-mortem examination showed a nodule of tumour about 1 grm. in weight in the right axilla. The chest-wall was bulged out in all directions, and, when opened, almost the whole of the right lung and the upper lobe of the left were seen to be replaced by masses of new growth. The liver was firmly adherent to the diaphragm, and a small white nodule was lying on the outer aspect of the liver. The mouse was preserved entire in Zenker's fluid, and, after decalcification and imbedding in paraffin, was cut in serial sections through an oblique antero-posterior plane. The recurrent growth in the right axilla contained carcinomatous and sarcomatous elements, and their distribution in the metastatic deposits could be followed very accurately in the serial sections. The metastatic deposits in the lungs were also mixed, the carcinomatous part preponderating over the sarcomatous (*vide* Fig. N). Emboli of sarcoma cells as also of carcinoma cells were present in several branches of the pulmonary artery, and one large branch was almost entirely occluded by a mass of actively dividing sarcoma cells. A further invasion of the systemic circulatory system had taken place from the lung, but only carcinoma cells had been transported. Several branches of the mesenteric artery were completely or partially occluded by carcinoma cells, and in some the growth had passed through the vessel wall. At two different places the submucous coat of the small intestine was the seat of relatively large metastases. The upper pole of the right kidney was entirely replaced by a large growth, and the left kidney contained several smaller growths. Cancerous embolism of several branches of the renal artery were also seen, and the lower pole of the

right kidney was the seat of a large anæmic infarct at the apex of which a medium-sized artery was found with lumen completely occluded by cancer cells. The nodule of tumour on the outer surface of the liver was completely isolated by a secondary abscess formation resulting from a localised gangrenous area of the intestine. All the abdominal metastases were alveolar in type with a very delicate stroma. In none of the sections was there any evidence of an invasion of the systemic system by the sarcoma cells.

A third mouse of series 13c was also operated upon on two occasions, each time showing pure carcinoma. After the second operation the recurrence was allowed to grow for sixty-one days, and then the animal was killed. The histological investigation of this tumour revealed abundance of sarcomatous tissue. Metastases in the lungs of this mouse were pure carcinoma.
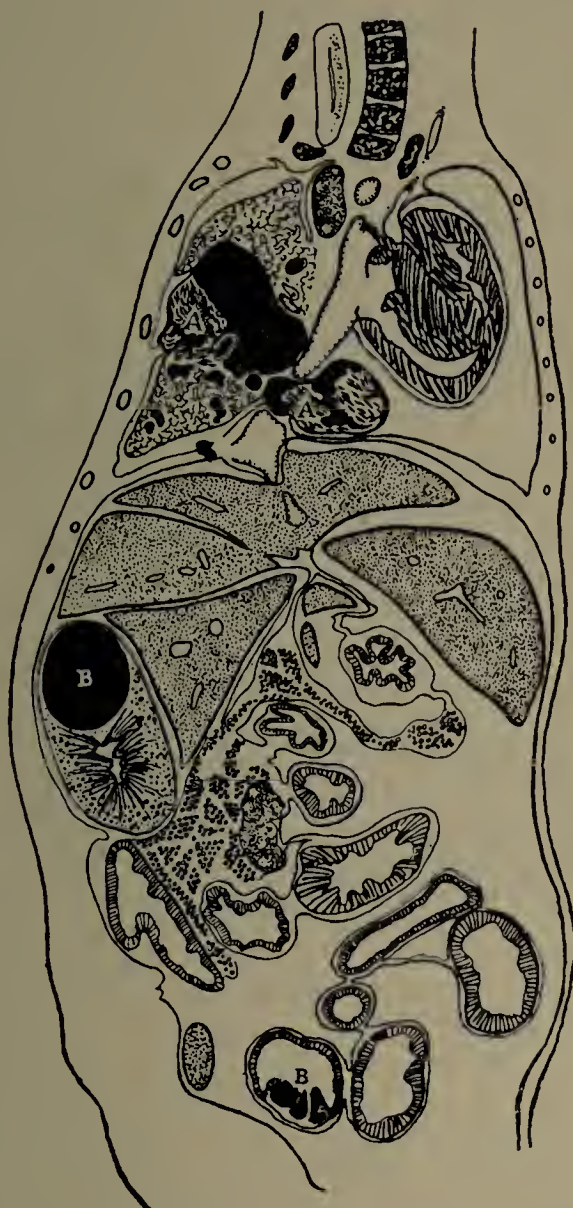

Fig. N.—100/13c, No. 10. Oblique sagittal section of whole mouse, showing mixed metastases in lungs A, pure carcinomatous metastases in right kidney and small intestine B. (× ¾).

A fourth tumour of 13c which had been growing progressively for sixty-nine days was also transplanted, giving 14p. In part it was extremely hæmorrhagic in character, but another part showed over a fairly large area sarcomatous transformation of the stroma.

A parallel strain to 13c was made by transplanting a second tumour of 12b into series 13h (*vide* Fig. M). It was 56 days old, but macroscopically no evidence of growth was seen until twenty-eight days before the subsequent transplantation. Microscopically, it had a minimal amount of stroma (Plate XIX. Fig. 4), which, when examined in " early stages," degenerated and disappeared (cf. Plate XX. Fig. 7). In addition to examining " early stages," four mice were killed at twenty-one days and their tumours preserved entire in Zenker's fluid. Two tumours were transplanted, the first at thirty-one days giving 14i. The

subsequent behaviour of the daughter tumours of 13H to 14I are also given in Fig. M, where it will be seen that it is similar to 12B to 13C.

A second tumour of 13H was operated upon thirty-eight days after inoculation, and was transplanted, giving series 14O. Its stroma then showed no sarcomatous change. Forty days later the recurrent tumour was excised and examined, when one or two small areas in the centre of the tumour were seen to be distinctly sarcomatous. Instead of the usual delicate collagen fibrils with here and there a small oval fibroblast, there were present several small collections of spindle-shaped cells arranged in columns, and showing numerous mitotic figures. A third operation was performed forty-two days after the second, and this time no evidence could be detected of any sarcomatous change in the stroma. A month later a fourth operation was performed, and this time sarcomatous tissue was again present. This mouse is still under observation with a recurrent tumour in the right axilla.

Three additional tumours of series 13H were allowed to go on growing without interference for a long period in one animal. The first was examined
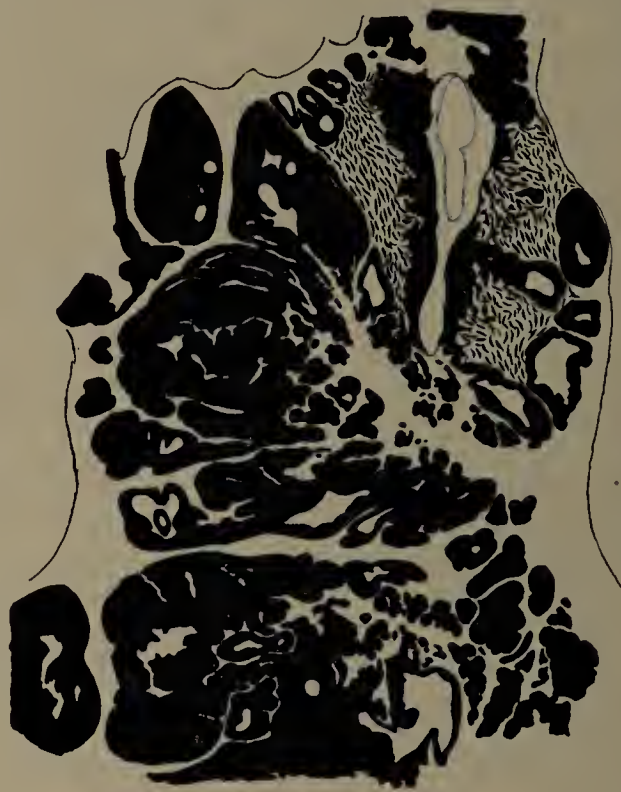

FIG. O.—100/13H–14Y. Tumour, aged 60 days, showing sarcomatous change restricted to one end of tumour. (× $\frac{5}{4}$.)

at fifty-nine days, and was found to be a mixed tumour. The relationship of the sarcomatous and carcinomatous tissues was here very intimate, the former being disposed like a supporting tissue around the irregularly shaped islands of carcinoma. "Early stages" of this tumour were examined, and twenty hours after inoculation the sarcomatous and carcinomatous components were seen growing side by side. The second tumour was examined on the sixtieth day, and the distribution of the sarcoma is shown in Fig. O. The third tumour was allowed to go on growing for eighty-four days. When examined it was found that the sarcomatous tissue was in the preponderance over the carcinomatous in the proportion of about 4 to 1.

To recapitulate, in this series 13H, six young tumours retained morphologically their pure carcinomatous character, and three old tumours showed the morphological characters of mixed tumours. The tumour of 13H which gave rise to 14O gave in the fifteenth generation two tumours of a mixed character so soon as thirty-eight days after transplantation. This is the earliest date at which the change has yet been seen in tumour 100.

Reverting to the daughter tumours of series 12B (Fig. M), a third tumour was examined and transplanted. It presented peculiar features, inasmuch as for the first five weeks after inoculation no growth of the implanted graft

could be detected. Then a small nodule about the size of a pinhead could be palpated. It remained this size for a month, and then began to grow progressively. One hundred and two days after inoculation, and thirty-five days after the onset of growth, the tumour was extirpated, and the material inoculated into twenty mice, giving series 13s. Microscopically, the growth was a pure carcinoma. The tumour recurred, grew rapidly, and was again excised thirty-three days after the first operation. Transplantation was again done into twenty mice, giving series 13u, and two large slices through the longest diameter of the tumour were preserved and examined microscopically. The recurrent growth showed large areas of sarcomatous tissue, mingled with the epithelial parenchyma. The subsequent behaviour of the daughter series of this strain is also given on Fig. M. Since it conforms to the type of those already described there is no necessity to give a detailed description. The sub-transplantations of series 12g (*vide* Fig. D) do not require to be specially discussed. They behave in the same manner.

General Consideration of Sarcoma Development in Tumour 100.

The experiments which have been detailed above enable us to draw certain conclusions regarding the development of sarcoma in this propagable hæmorrhagic adeno-carcinoma. The behaviour of the stroma of the pure carcinomata has been followed in all its stages, and also its behaviour upon transplantation into fresh animals. In addition to the examination of " early stages," a histological examination has been made of three or four tumours from all the later series at about the thirtieth day of inoculation, whilst a similar number have been allowed to go on growing in the same animal for a longer period. About 500 tumours have been examined, in addition to those given in the genealogical tree, as having been further transplanted.

The behaviour of the stroma of this tumour is of especial interest, and it has received a great deal of attention since a sarcomatous change was first observed. An examination of the site of inoculation has been frequently carried out for the pure carcinomata, for the mixed tumours, and for the pure sarcomata. When the grafts of a pure carcinoma are examined twenty hours, forty-eight hours, etc., after inoculation, it has been found that the epithelial cells continue to divide, but that the introduced stroma degenerates. No evidence of a survival after transplantation of any of the connective tissue elements has yet been detected. From the third to the fourth day a stroma is provided by proliferation of the tissues of the new host. The degree of vascularity of this new stroma varies greatly ; at one time the angioblasts are predominant in the reaction, and at another time the fibroblasts.[1] The character of the stroma of tumours of

---

[1] The nature of the connective tissue and vascular scaffolding supplied by a new host has been shown to be specific for different tumours by Bashford, Murray, and Cramer (1905), who referred it to the chemiotactic influences exerted by the tumour cells themselves on the fibroblasts and angioblasts respectively. The same conclusion was later arrived at by Ehrlich (1906) on evidence of a different nature.

about thirty days' growth also varies greatly in different generations, and in different series of the same generation. In most tumours it is rather scanty, and consists of delicate bundles of collagen fibrils with comparatively few fibroblasts. As the tumours become older the connective tissue becomes very œdematous, and the blood vessels dilate to form large blood sinuses. Through thrombosis of some of these sinuses large areas of parenchyma and stroma are deprived of their nourishment and degenerate as described by Gierke (1906 [15. 16]), for this group of transplantable hæmorrhagic tumours. The stroma in the neighbourhood of such necrotic areas often increases in cellularity, due partly to an infiltration by small round cells and plasma cells, but also partly to a proliferation of the fixed connective tissue cells. This increase of cellularity of the stroma in the neighbourhood of necrotic areas is quite distinct from the sarcomatous change, and indeed it has been usually found that extensive necrosis delays the onset of the sarcomatous change, inasmuch as it involves the death of the older portions of the tumour. Some of the carcinomatous tumours of 100 show a fairly abundant and cellular stroma when they are quite young, which again is quite distinct from the sarcomatous stroma histologically, and, what is of greater importance, does not survive transplantation. In the daughter tumours it may again give place to the most delicate stroma.

The transformation in the character of the stroma which leads to the development of sarcoma in the very old tumours of 100, begins first over a very small area towards the centre of the tumour. It affects solely the fibroblasts of this area, which begin to divide very actively, and rapidly produce a mass of cells very uniform in type. For the majority of tumours the sarcomatous change in the stroma has been unicentric, in some, however, it has been pluricentric. For tumour 100 the cells in the area or areas exhibiting the change are usually spindle shaped, and by repeated and active division they spread rapidly throughout the central part of the tumour, penetrate into and break up the carcinomatous part, and finally replace it. They differ fundamentally from the stroma cells previously considered. Histologically, the tumour which they build up is a sarcoma, capable of entering the blood stream and producing metastases in distant organs as already described by Haaland for Ehrlich's case (1906 [18]), and also for our tumour " 37 " (1908 [19]). When transplanted into a fresh host they continue their growth and divide actively. By allowing a carcinomatous tumour of 100 to remain over a long period in one animal, a type of stroma is obtained which survives transplantation, which has a high proliferative power, which is endowed with the capacity of infiltrative growth, and, in short, is endowed with all the properties of a malignant new growth, namely, a sarcoma.

This change has been seen at the fiftieth day, and exceptionally at a still earlier date, but it has been seen most frequently between

the fifty-fifth and sixtieth day. The number of tumours which have now shown this change is about a hundred, and there have been found only four or five exceptions to the rule that sixty days of sustained growth in one animal is sufficient to produce a sarcomatous change in the stroma of this carcinoma.

From a general survey of the behaviour of carcinoma 100 in different strains and in different generations, one has received the impression that the change in the stroma is induced earlier at one time than at another. In some series the onset of the sarcoma would not be detected until after seventy days' growth, whilst in other series the same change was already present as early as after thirty-eight days of active growth. This difference cannot be attributed to differences in individual mice, for the onset of sarcoma development is uniform for all the mice of any series.

The actual area of stroma over which this change takes place is at first very limited. Tumours have been examined in which the change was limited to an area of a few millimetres in diameter, and, whilst discernible in one slice through the tumour, may not be detectable in the next parallel slice. The number of mitotic figures present in these small areas is relatively enormous, and the smallness of the total sarcomatous area points to its very recent origin. Very old tumours, seventy to eighty days old, may show great preponderance of sarcoma over carcinoma, but as yet only one tumour has been seen which had advanced from pure carcinoma at the time of inoculation to the stage of total elimination of the carcinoma by sarcoma in the one animal. The number of instances in which it is possible without operation to prolong the stay of a growing tumour in one animal for much longer than sixty days is, however, limited, and no evidence exists against the assumption that this tumour would regularly lead to the development of a pure sarcoma in one animal after a sufficient lapse of time.

The table appended gives the result of the operations which were performed upon seven mice of different series, which had been inoculated with grafts of pure carcinoma. In all seven the duration of the growth of the tumour in the one animal has been a long one, and yet in two of the mice no development of a sarcomatous stroma had taken place at the sixtieth day. This apparently contradictory result does not, however, quite upset the view, based upon the previous experiments, that prolonged sojourn of a tumour in one animal suffices to induce sarcomatous transformation of the stroma of the tumour. It only serves to modify it in the direction that prolonged contact of the parenchyma of tumour with the same stroma is what is necessary. When a tumour is operated upon, only a small fragment at the periphery about 0·02 grm. is left behind. In the subsequent development of this fragment to a tumour weighing about 4 or 5 grms. a large supply of new stroma is required. The actual proportion of this

stroma which is supplied by the stroma left behind with the fragment of tumour cannot of course be estimated, but it is most probable that the vast majority of the new stroma cells are derived from the sur-

Series.	Date of Inoculation.	Operation.	Days after Inoculation.	Histology of Excised Tumours.
10c, No. 9	Sept. 9, 1908	Growth began, Nov. 30, 1908	63	
		First operation, Jan. 15, 1909	108	Carcinoma, stroma cellular.
		Second operation, Feb. 4, 1909	127	Small area of sarcoma, *vide* Fig. F.
		Mouse killed, Mar. 31, 1909	182	Sarcoma abundant, *vide* Fig. G.
12b, No. 5	Nov. 30, 1908	Growth began, Jan. 28, 1909	59	
		First operation, Dec. 3, 1909	102	Carcinoma, stroma cellular.
		Second operation, Apr. 14, 1909	135	Large areas of sarcoma.
		Mouse died, Apr. 15, 1909	136	
12e, No. 9	Dec. 31, 1908	First operation, Jan. 28, 1909	28	Carcinoma.
		Second operation, Feb. 20, 1909	51	,,
		Third operation, Mar. 17, 1909	76	,,
		Fourth operation, Apr. 1, 1909	91	,,
		Mouse killed, May 1, 1909	121	,,
12e, No. 17	Dec. 31, 1908	First operation, Feb. 22, 1909	53	Carcinoma, stroma cellular.
		Mouse died, Apr. 14, 1909	104	Small areas of sarcoma.
13c, No. 10	Jan. 7, 1909	Growth began, Jan. 28, 1909	21	
		First operation, Mar. 3, 1909	55	Carcinoma.
		Second operation, Apr. 7, 1909	90	Small areas of sarcoma.
		Mouse killed, Apr. 28, 1909	111	Sarcoma abundant.
13c, No. 14	Jan. 7, 1909	First operation, Feb. 19, 1909	43	Carcinoma.
		Second operation, Mar. 19, 1909	71	,,
		Mouse killed, May 19, 1909	132	Sarcoma abundant.
13h, No. 14	Jan. 25, 1909	First operation, Mar. 4, 1909	38	Carcinoma.
		Second operation, Apr. 13, 1909	78	Small areas of sarcoma in centre.
		Third operation, May 25, 1909	120	Pure carcinoma.
		Fourth operation, June 21, 1909	147	Mixed tumour.

rounding tissues recently laid bare by the knife. That is, the stroma of the recurrent tumour has not been previously associated with the carcinomatous cells, and the process of sarcoma development is in this sense actually retarded by the operative interference. The varying quantity of old stroma in the recurrent tumour is the most probable explanation of the inconstant results of operative interference.

As regards the general biological behaviour of the tumours during and after the sarcomatous change, many interesting facts have been noted. The rate of growth and the transplantability of the carcinomatous tumours have been already described, but the sarcomatous tumours derived from these carcinomata behave very differently on transplantation. Ten days after inoculation of the sarcomatous graft, tumours are obtained of a size which is only seen in the carcinoma after about three to four weeks. Haaland found that the sarcomatous tumours of strain 37 grew more rapidly than the carcinomatous tumours from which they were derived, and the same phenomenon has been exhibited by strain 100. The growth curves published by Haaland (1908 [19]) can be compared with the size of tumours obtained in the sarcoma and carcinoma tumours of 100 as shown on Fig. P. Columns one and two of Fig. P give the sizes of the sarcoma tumours of tumour 100, ten and seventeen days after inoculation, and in columns three and four the same is given for a strain of 100 carcinoma. Such enormous differences as these are incomprehensible on the assumption that there is present merely a histological rearrangement of cells, and not the development of a new variety of tumour. A higher percentage of tumours is also obtained with the sarcomata, but they differ again from the carcinomatous tumours in that spontaneous absorption is more common. It is rare that a carcinomatous tumour which has attained to a weight of about 1 grm. becomes smaller until it finally disappears, whereas this phenomenon occurs in the majority of the sarcomatous tumours of 100. Mice which have thus cured themselves of sarcoma 100, are negative to the subsequent inoculation of carcinoma 100. In reference to the question as to whether this cure is secondary to a self-immunisation, or the immunisation is secondary to the cure of the tumour by absorption, experiments described by Bashford, Murray, Haaland, and Bowen (1908 [12]) point to the former being the more likely.

When the sarcomatous change has started in what was previously a pure carcinoma, there is no alteration in the speed of growth. Those tumours which have been propagated as mixed tumours through one or more generations, retain the carcinomatous rate of growth in the sub-transplantations. When the sarcoma cells have become quite freed from the carcinoma cells, their rate of growth is increased very much. Therefore it has to be assumed that the carcinomatous cells are capable of exerting a restraining influence upon the growth

FIG. P.—Exp. 100/14₂ᴊ. Mice 1–17, all inoculated in right axilla with
0·015 grm. of pure sarcoma on June 22, 1909.
Exp. 100/20ᴀ. Mice 18–34, all inoculated in right axilla with 0·015
grm. of pure carcinoma on June 21, 1909.

of the sarcoma cells, even although the latter be more numerous and ultimately replace the carcinoma cells entirely.

The objection has been raised by von Hansemann (1905 [20]) and Schlagenhaufer (1906 [27]), that those tumours, which during propagation have given rise to the development of mixed tumours, may have contained sarcomatous cells in the primary growth. In tumour 100, examination of the primary and recurrent growths, as also of the metastases in the lungs, does not reveal the presence of any sarcomatous tissue. The view that the latter may have been lying latent from the primary tumour onwards, but not detectable morphologically, is scarcely to be reconciled with the whole behaviour of the tumour during propagation. As late as the seventeenth passage, pure carcinomata were obtained of which the grafts were capable of producing mixed tumours in any mouse where active growth had gone on for about sixty days. Each of these grafts represents about one-fortieth of the total bulk of the tumour from which it is taken, and if this subdivision be carried back seventeen times the fraction of the primary growth represented in this graft is infinitesimally small. Further, the hypothesis that sarcomatous cells existed in the primary tumour, and are represented by their lineal descendants in a seventeenth generation of sub-transplantation, makes it also necessary to assume that they have been during the continued propagation in a state of very active division, so as to have become equally distributed throughout every part of every tumour. Only by means of this additional assumption can the presence of sarcoma cells in every tumour of the seventeenth generation be reconciled with the presence of sarcoma in the primary tumour. Their presence in this way would remove them from the field of speculation and place them again in the field of morphology, and it is upon morphological evidence that the validity of this assumption must be decided, and, as has been demonstrated, on these grounds there is no evidence of their presence.

Another point which requires to be mentioned is the possibility of the derivation of the spindle cells of the sarcoma through morphological alteration of the epithelial cells. The epithelial cells of carcinomatous tumours may assume a spindle shape and resemble sarcomatous tissue as described by Apolant (1908 [4]), and illustrated by Haaland (1908 [19]) for tumour 37, and the same is occasionally seen in tumour 100. When the development of sarcoma is observed at its inception, the process is strictly limited to the stroma of a localised area of the tumour, and the sarcomatous tissue is sharply demarcated from the carcinomatous alveoli and acini. Later, when the sarcoma cells begin to invade the alveoli, microscopical pictures may be obtained in which a strict distinction between epithelial and sarcomatous cells can no longer be carried out, but in most cases the distinction is quite marked, as shown in Plate XIX. Fig. 5, Plate XX. Fig. 9. The subsequent growth of the sarcomata, and their parallel behaviour to the

spontaneous transplantable sarcomata of the mouse, serve also to consolidate the views based upon morphological grounds only. Krompecher (1908 [21]) holds, as the result of a morphological consideration of mixed tumours as occurring in man, and of a casual examination of preparations of Ehrlich's mixed mouse tumours, that the only possible explanation of the sarcoma development is a transformation of the epithelial cells into cells histologically of a sarcomatous type. This view is based entirely upon the changes seen in the later admixture of carcinomatous and sarcomatous cells which we have found to be purely a secondary phenomenon, and, as it leaves entirely out of account the fundamental changes in the biology of the tumour, it cannot be regarded as being even a remotely possible explanation. Borst (1909 [14]) also inclines to the view that the carcino-sarcomata of the mouse are produced by an alteration of the parenchyma of the carcinoma into sarcomatous looking tissue, but he only refers briefly to the question.

The first case of sarcoma development in this tumour was seen at the twelfth *passage*, but could also be demonstrated in a tumour of the eleventh *passage*. Is this property of the tumour to be regarded as primary, or must it be regarded as an acquirement through repeated propagation ? To this question no definite answer can be given, as the prolonged stay in one animal, subsequently demonstrated to be requisite for the development of sarcoma in tumour 100, was not consummated in any of the earlier transplantations. There exist no tumours of the earlier generations which grew actively for sixty days in one animal. The age of the primary tumour is unknown, but the period of active growth of its recurrence is, and did not approach to sixty days. In the earlier part of this paper, stress was laid upon the fact that a morphological and a biological analysis of the primary and transplanted tumours gave no basis for the view of any progressive alteration in the character of the tumour as the result of repeated transplantation. It is impossible to say that the carcinoma cells only acquired the power of producing sarcomatous transformation of its stroma in consequence of biological changes arising through successive *passage*. The opposite view, that the primary tumour possessed the power, but had never had the chance of exercising it, is much more likely. Spontaneous mixed tumours of the mouse mamma have been described, so that there is evidence in support of the latter hypothesis.

At first view it appears very striking that a pure carcinomatous tumour should give rise in one animal to sarcomatous tissue capable of running on rapidly to elimination of the carcinoma, and serious reflection might lead to the assumption that the process was peculiar to experimentally propagated carcinomata, and played no part in the biology of malignant new growths as observed in man. This, however, is not so, and Apolant (1906 [3]) has recorded a case observed

by Schmorl, which ranks as one of the most interesting examples of the occurrence of a similar process in the human subject.

The case is that of a woman operated upon by Thiersch on two occasions for a tumour of the thyroid gland. At the first operation the tumour was an adenoma with suspicion of carcinoma. The tumour recurred, was operated upon a second time, and was then found to be a carcinoma with sarcomatous stroma. At the post-mortem examination the recurrent tumour, as also the metastatic deposits, proved to be pure sarcomata. An analogous case, reported by Schmorl at the April meeting of the Deutsche Pathologische Gesellschaft 1908, was that of a man who had died from carcinoma of the prostate. Numerous secondary deposits were present in the skeletal system, and those in the head of the right femur were surrounded by an extremely cellular mesoblastic tissue of a sarcomatous type. Secondary deposits in the lungs were pure sarcomata. This case has subsequently been described in full detail by Reichmann (1909 [26]). Schmorl interprets the case as one where the carcinomatous cells had induced a malignant change in the tissues of the femur, which had in turn produced metastatic deposits in the lungs. Carcinomata of the prostate are notorious for producing secondary deposits in the skeletal system, and have a very great influence upon the structure of the bones which they invade, leading, as they do, to the development of bony tissue in the stroma of the secondary nodules. Axhausen (1909 [6]), who has recently worked through a series of cases of osteoplastic carcinomatosis, emphasises the view, first enunciated by von Recklinghausen, but in a tentative manner, that this osteoplasia is the direct result of the irritation of the stroma by the cancer cells. This irritation he presumes to be chemical in nature, and he further states that a certain period of time is requisite to allow of this irritation producing the above-mentioned effect. In Schmorl's case, the irritation of the cancer cells had produced a malignant change in the stroma, parallel to the change observed in the various transplanted tumours of tumour 100. Krompecher also had the opportunity of examining a tumour of the orbit in man which had been operated upon and had recurred. Microscopically, the tumour material from the first operation was typically carcinomatous with a "mucoid" stroma. In the recurrent tumour, the carcinomatous part occupied only a limited area, whilst the rest of the tumour had the structure of a typical spindle-celled sarcoma.

The development of sarcoma has now been followed in tumour 100 for about ten months, which, although almost equal to a half of the total duration of the life of a mouse, is only a brief period in the life-history of a propagated tumour. During these ten months the tumour has behaved very consistently in regard to its power of inducing sarcoma development, but the possibility of it subsequently altering its behaviour must not be left out of account. It has been shown that biologically and morphologically the other features have shown only subordinate changes, and this fact leads one to expect that the future sarcoma development will take place in a manner similar to those cases, about a hundred in number, already observed in this tumour.

NATURE OF SECONDARY SARCOMA DEVELOPMENT.

When one comes to a consideration of the causes which may be made responsible for the sarcomatous changes in the stroma, there are

three main views already enunciated which require to be reviewed. One view is, that when tumours are transplanted, not only does the parenchyma grow, but the stroma elements also continue to divide. Repeated passage of such transplantable stroma cells, and the irritation resulting therefrom, may be made responsible for the onset of the sarcomatous change. This view was first advanced by Apolant and Ehrlich, but appears subsequently to have been dropped, as there was no evidence that their tumours were exceptions to the rule, previously laid down by Bashford, Murray, and Cramer, that the stroma of the ordinary transplantable carcinomata of the mouse degenerates on transplantation. The possibility of transplantable stroma cells being a contributory cause was again revived by Haaland for tumour 37. Haaland found that certain strains of this tumour, previous to development of sarcoma, did not behave quite like the other carcinomata ; the possibility that certain of the stroma cells were able to survive the transplantation, and to grow in the new host could not be excluded. When the tumours of 37 were allowed to grow for a very long period in one mouse, no sarcomatous changes in the stroma were detectable, excepting in one doubtful case (37/2c), but in other strains (37/9B), after a certain number of *passages*, the cells did alter in the direction of acquiring malignant new properties. Haaland regards as a possible explanation of the development of sarcoma in tumour 37, that certain of the cells of the stroma survive transplantation, but are not yet endowed with neoplastic characters. After a varying number of passages, during which they have been thrown into an intermittent state of regeneration, and during which the repeated transplantation has allowed of a certain degree of selection, the descendants of the cells which originally survived transplantation give rise to a new race of cells, sarcoma cells. In this manner the gap between granulation-tissue cells and sarcoma cells may be bridged over.

That tumour 100 does not behave quite as does tumour 37, is only another instance of the multiplicity of ways in which new growths may arise. The behaviour of the stroma of tumour 100 on transplantation has been studied in a great many series, and no evidence has been obtained strong enough to allow of this tumour being made an exception to the general rule that stroma degenerates on transplantation. Repeated transplantation of the tumour also cannot be made directly responsible for the onset of the sarcoma change. Any representative carcinomatous tumour of 100, about thirty days old, may be taken and transplanted through five, six, or more generations, and yet no sarcomatous change be observed. Should, however, a tumour from any one of these generations be allowed to grow sixty or more days in one animal, sarcomatous changes in the stroma will almost certainly be seen in some part of the tumour.

Full attention has been paid to the possibility of sarcomatous tissue being present in such a series of apparently pure carcinomata,

and that the hurried passage did not give the sarcoma elements time to develop. A glance at Plate XIX. Fig. 4, from a pure carcinoma of the thirteenth generation, will be sufficient to demonstrate that in this tumour there can be little possibility of sarcoma elements being present, and yet in the next generation of this very tumour three old daughter tumours showed sarcoma development, whilst five young daughter tumours remained pure carcinomata.

In infective diseases the period elapsing between infection and onset of symptoms varies enormously in different diseases, and the criticism may be anticipated which, based upon a false analogy, asserts that here we are only dealing with an instance of the difference in the "incubation period" of two distinct morbid processes. Reasons have already been given for the belief that the tumour we start with is a pure carcinoma, and that it is inconceivable how "latent" sarcoma cells can be transferred with each transplantation. When grafts of the mixed tumours are examined twenty hours after inoculation (*vide* Plate XX. Fig. 6), the sarcoma and carcinoma cells are seen growing side by side, and the adult tumours, 20 to 40 days old, developed from such mixed grafts, contain sarcoma or carcinoma cells in varying proportions, but always with the tendency towards elimination of the latter. This observation throws the hypothesis of a difference in incubation period quite out of court.

Repeated transplantation of 30-day old pure carcinomatous tumours fails in tumour 100 to induce sarcoma development, each time the old stroma dies and a new one is provided. When a 60-day old growth is transplanted, some parts of its stroma, already showing the morphological characters of sarcoma, survive transplantation, and are present in the young daughter tumours. Between the thirtieth and the sixtieth day, parts of the stroma alter from being normal connective tissue cells, and become sarcoma cells.

Another standpoint from which sarcoma development may be viewed is that of the carcinoma cells inducing malignant changes in the stroma cells. This possibility was entertained strongly by Ehrlich and Apolant, who attached importance also to an increase of the "virulence" of the carcinoma cells. They stated that the sarcoma development occurred when the tumours were growing very rapidly, and assumed that the sarcoma development was due to an excessive irritation of the stroma cells produced through some chemical altera- tion in the carcinomatous cells. Haaland did not observe that the sarcoma development took place when the tumours were growing very well; if any change in growth could be observed when the sarcomatous change was taking place, it was that the tumours grew badly.

Similarly, we have noted that tumours, exhibiting the maximal rate of increase for this tumour 100, were not liable to give sarcomata at an earlier date than more slowly growing tumours. The theory

that rapid growth of the epithelial cells is necessary to produce sarcoma development does not apply generally. There are a great many rapidly growing propagable carcinomata which have not yet shown any evidence of giving sarcoma, and the influence of the carcinoma cell upon the stroma cell, so that the latter may become malignant, must therefore be qualitative not quantitative. The mouse carcinomata differ among themselves as regards their relation to the experimental production of sarcoma, and where the epithelial element is the only one which is carried over from generation to generation, it seems difficult to escape the conclusion that peculiarities of the parenchyma are responsible for the occurrence : at the same time it is apparent that rapidity of proliferation is not the quality of the parenchyma responsible for the change. Tumour 100 is very suitable for defining more exactly than has hitherto been possible the degree to which the carcinoma cell can be made answerable for the change. So far as this tumour has yet been observed, it would appear that the stroma cells must be in contact with the carcinoma cells for a fairly long period. Several instances have been observed where the tumours were present in the one animal for about eighty or a hundred days, but where microscopical evidence of growth did not set in until the last twenty or thirty days. In such cases as these, sarcoma development has *not* taken place, showing how closely associated the sarcoma development is with the progressive continuous growth of the epithelial cells. Mere contact of the stroma cells with the epithelial cells is not sufficient; the components must be in a state of growth and division to produce the transformation of the stroma.

That certain mice may be predisposed to sarcoma development, and may react to a stimulus by the formation of an excessive amount of mesoblastic tissue, has been put forward by Ehrlich as a possible factor in sarcoma development, who cites the well-known fact that individual variations exist in the rate of healing of simple wounds, and in the amount of scar tissue which is produced. In certain individuals this latter may be so excessive as to be almost a neoplasm, as in the "keloid" condition. The assumption of such a contributory factor is quite superfluous in the case of tumour 100, as with this tumour practically every mouse is a "keloid" mouse, and such a generalisation renders this explanation nugatory. Jensen's tumour has been transplanted probably into more mice than any other tumour, and yet it has never been inoculated into a "keloid" mouse.

All the observations which have been made upon tumour 100 point to the view that, in this case, the epithelial cells of the tumour are responsible for the induction of malignancy in the stroma cells. It does not appear to be advancing the question much to assume that these changes are produced by chemical or physical means. The only methods by which we are at present able to analyse the change are biological, and it appears more logical to draw the con-

clusion that the change is a vital one, and closely bound up with the other vital changes taking place in the cells.

From a physiological point of view, are these cases of sarcoma development to be regarded as an attempt on the part of the animal to rid itself of the new growth? Where the change takes place in a tumour, the tendency is always towards elimination of the carcinomatous cells. Spontaneous cure of transplanted carcinomata is frequent, but it differs fundamentally from sarcoma development. When spontaneous absorption of a tumour is taking place there is a primary degeneration of the carcinoma cell, but where sarcoma development occurs the carcinoma cell is in a state of activity.

Apolant (1908[4]) has suggested that the natural termination of all carcinoma may be sarcoma development, and that the apparent immortality of malignant epithelial tumours is not real. Apart from the fact that many tumours which have been propagated over long periods, *e.g.* Jensen's tumour, propagated since 1901, have not shown sarcoma development, there are other phenomena observed which speak against such a conception of sarcoma development. Tumour 37, which has frequently given sarcomata, is still being propagated in many strains as pure carcinoma, and it has been shown for tumour 100, where the sarcoma change has taken place over part of the tumour, that it is possible to isolate fragments of pure carcinoma which can by suitable methods be further propagated as pure carcinoma for an indefinite period. The sarcomatous change is therefore to be regarded as something incidental which is not essential to the growth of all carcinomatous tumours. Whether other tumours may behave in a manner similar to tumour 100, and give sarcomata if they be left for long periods in one animal, or behave as tumour 37, cannot yet be decided. Several different tumours have been already investigated to ascertain whether the very old tumours tended to become mixed tumours, but so far with negative results. This is quite in keeping with the behaviour of neoplasms in man, where the vast majority of epithelial tumours remain as such over long periods, until death has stopped all further growth.

Summary.

1. During the propagation of a hæmorrhagic adeno-carcinoma of the mamma of the mouse, a change in the character of the stroma has been observed.

2. This change consists in the acquirement of neoplastic properties by what were previously normal connective-tissue cells of the stroma of the tumour.

3. Continuous growth of the epithelial cells of this tumour in one animal over a long period of time, usually about fifty-five days, appears to be the factor determining the malignant transformation of the stroma cells.

4. Once the sarcomatous change has been initiated it always tends to the entire elimination of the carcinomatous component, and the resulting tissue can then be propagated indefinitely as pure sarcoma.

5. By shortening the duration of growth in each animal, it is possible to maintain the propagation of strains of pure carcinoma, and from these pure strains mixed tumours can be obtained at will.

6. Lastly, a survey of all the facts leads to the conclusion that the epithelial cells of tumour "100," when inoculated into a fresh host, can induce a neoplastic transformation of the connective tissues of this host.

REFERENCES.

1. EHRLICH AND APOLANT . "Beobachtungen ueber maligne Mäusetumoren," *Berl. klin. Wchnschr.*, 1905, S. 871.

2. ,, ,, . "Weitere Erfahrungen ueber die Sarkomentwicklung bei Mäusecarcinomen," *ibid.*, 1906, S. 37.

3. APOLANT . . . . . . "Die epithelialen Geschwülste der Maus," *Arb. a. d. König. Inst. f. exper. Therap. zu Frankfurt-a-Main*, Jena, 1906, Heft 1, S. 7.

4. ,, ... "Ueber die Genese des Carcinoms," Histologischer Teil, *Verhandl. d. Deutsch. path. Gesellsch.*, Jena, 1908, 12 Tagung, S. 3.

5. ,, ... "Ueber künstliche Tumormischungen," *Ztschr. f. Krebsforschung*, Berlin, 1908, Bd. vi. Heft 2, S. 251.

6. AXHAUSEN . . . . . "Histol. Stud. ueber die Ursachen und den Ablauf des Knochenumbaus im osteoplastischen Karzinom," *Virchow's Archiv*, 1909, Bd. cxcv. S. 358.

7. BASHFORD . . . . . "Growth of Cancer: with Discussion," *Trans. Med. Soc.*, London, March 1905, vol. xxviii. p. 210.

8. BASHFORD, MURRAY, AND CRAMER "Action of Radium on Transplanted Mouse Tumours, and its Relation to the Spontaneous Arrest of their Growth," *Second Scientific Report of Imperial Cancer Research Fund*, London, 1905, p. 56.

9. ,, ,, "Infiltrative Extension and Metastasis Formation by Transplanted Malignant New Growths," *ibid.*, p. 38.

10. ,, ,, "Interpretation of the Differences and Variations in Transplanted Tumours of one Organ," *ibid.*, p. 52.

11. BASHFORD, MURRAY, AND BOWEN "The Experimental Analysis of the Growth of Cancer," *Proc. Roy. Soc. London*, 1906, vol. lxxviii. (B), p. 195.

12. BASHFORD, MURRAY, HAALAND, AND BOWEN "General Results of Propagation of Malignant New Growths," *Third Scientific Report of the Imperial Cancer Research Fund*, London, 1908, p. 262.

13. BORREL . . . . "Epithélioses infectieuses et epithéliomas," *Ann. de l'Inst. Pasteur*, Paris, 1903, tome xvii. p. 81.

14. BORST . . . . "Die echten Geschwülste," *Aschoff's Pathologische Anatomie*, Jena, 1909, Bd. i. S. 518.

15. GIERKE . . . . . . "Die Hämorrhagischen Mäusetumoren, mit Untersuch. ueber Geschwulstresistenz und Disposition bei Mäusen," *Beitr. z. path. Anat. u. z. allg. Path.*, Jena, 1908, Bd. xliii. S. 328.

16. ,, ... "The Hæmorrhagic Mammary Tumours of Mice," *Third Scientific Report of Imperial Cancer Research Fund*, London, 1908, p. 115.

17. HAALAND . . . . . . "Les Tumeurs de la Souris," *Ann. de l'Inst. Pasteur*, Paris, 1905, tome xix. p. 165.

18. ,, ... "Ueber Metastasenbildung bei transplantierten Sarkomen der Maus," *Berl. klin. Wchnschr.*, 1906, No. 34, S. 1119.

19. ,, ... "Contributions to the Study of the Development of Sarcoma under Experimental Conditions," *Third Scientific Report of Imperial Cancer Research Fund*, London, 1908, p. 175. Cf. also Bashford, Murray, and Haaland, "Ergebnisse der experimentellen Krebsforschung," *Berl. klin. Wchnschr.*, 1907, No. 39, p. 1238.

20. v. HANSEMANN . . . . . *Verhandl. d. deutsch. path. Gesellsch. zu Meran*, Jena, 1905, S. 169.

21. KROMPECHER . . . . . "Ueber die Beziehungen zwischen Epithel und Bindegewebe bei den Mischgeschwülsten der Haut und der Speicheldrüsen und ueber das Entstehen der Karcinosarkome," *Beitr. z. path. Anat. u. z. allg. Path.*, Jena, 1908, Bd. xliv. S. 88.

22. LEWIN . . . . "Exper. Beitr. z. Morphol. und Biol. bösartiger Geschwülste bei Ratten und Mäusen," *Ztschr. f. Krebsforschung*, Berlin, 1908, Bd. vi. Heft 2, S. 267.

23. LIEPMANN . . . . . . *München. med. Wchnschr.*, 1907, No. 27, S. 1345.

24. LOEB . . . . "Development of Sarcoma and Carcinoma after the Inoculation of a Carcinomatous Tumour of the Sub-maxillary Gland in a Japanese Mouse," *Med. Bull. Univ. Pennsylvania*, July 1906, vol. xix. p. 113.

25. MURRAY . . . . . . "Spontaneous Cancer in the Mouse," *Third Scientific Report of the Imperial Cancer Research Fund*, London, 1908, p. 69.

26. REICHMANN . . . . . "Kombination von osteoplastischer Carcinose mit Osteo - chondrosarkom, *Ztschr. f. Krebsforschung*, Berlin, 1909, Bd. vii. Heft 3, S. 639.

27. SCHLAGENHAUFER . . "Carcinom und Riesenzellsarkom derselben Mamma," *Centralbl. f. allg. Path. u. path. Anat.*, Jena, 1906, Bd. xvii. S. 385.

DESCRIPTION OF PLATES XVIII.-XXI.

PLATE XVIII.

FIG. 1.—Primary tumour 100. Shows acinous and alveolar parenchyma, with delicate œdematous stroma and dilated blood sinuses. Stroma and parenchyma in part necrotic. ($\times \frac{98}{1}$.)

FIG. 2.—Carcinomatous embolus of primary tumour 100 in pulmonary artery, showing also desquamation of endothelial lining of perivascular lymphatic space. ($\times \frac{145}{1}$.)

FIG. 3.—Primary tumour 100. Secondary nodule in lung, showing pronounced acinous structure with delicate stroma. ($\times \frac{154}{1}$.)

PLATE XIX.

FIG. 4.—(100/12B – 13H.)—Twenty-eight days old growth of carcinomatous tumour of thirteenth *passage*, showing alveolar character of parenchyma and delicate stroma. ($\times \frac{65}{1}$.)

FIG. 5.—(100/11B – 12F.)—Seventy-six days old tumour, showing sarcomatous transformation of the stroma, and active growth continuing in parenchyma and stroma. ($\times \frac{220}{1}$.)

PLATE XX.

FIG. 6.—Graft of mixed tumour, 100/11B – 12F, twenty hours after transplantation, showing sarcoma and carcinoma cells growing side by side. ($\times \frac{500}{1}$.)

FIG. 7.—Graft of pure carcinoma 100/13L, twenty hours after transplantation, showing degeneration of introduced stroma, and proliferation of carcinoma cells. ($\times \frac{495}{1}$.)

PLATE XXI.

FIG. 8.—(100/16A – 17B.)—Tumour, æt. 21 days, showing the almost pure spindle-celled character after three *passages* as pure sarcoma. ($\times \frac{500}{1}$.)

FIG. 9.—(100/16B, No. 8.)—Tumour, 76 days old, showing local overgrowth of the carcinoma by the sarcomatous stroma. ($\times \frac{40}{1}$.)
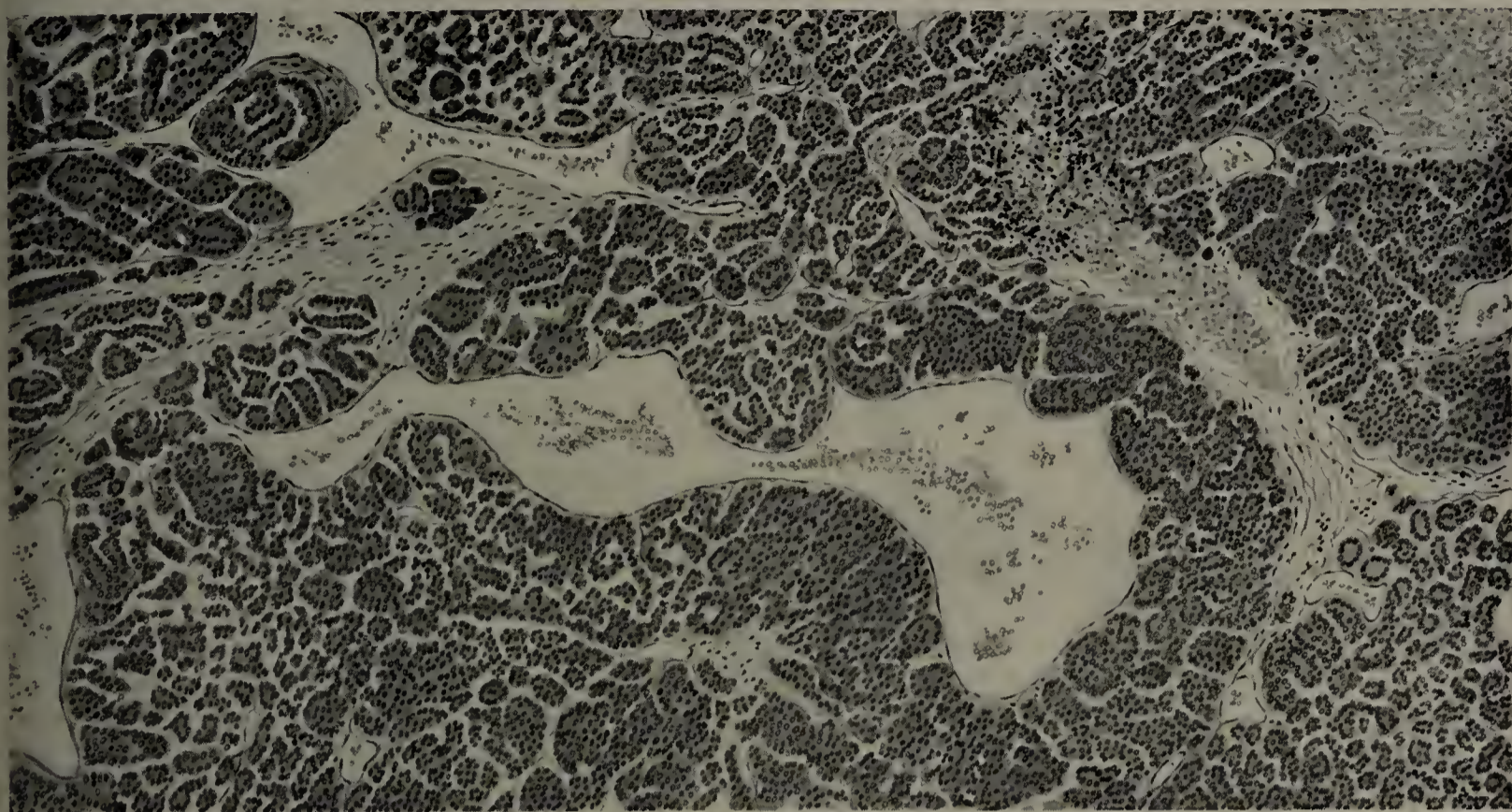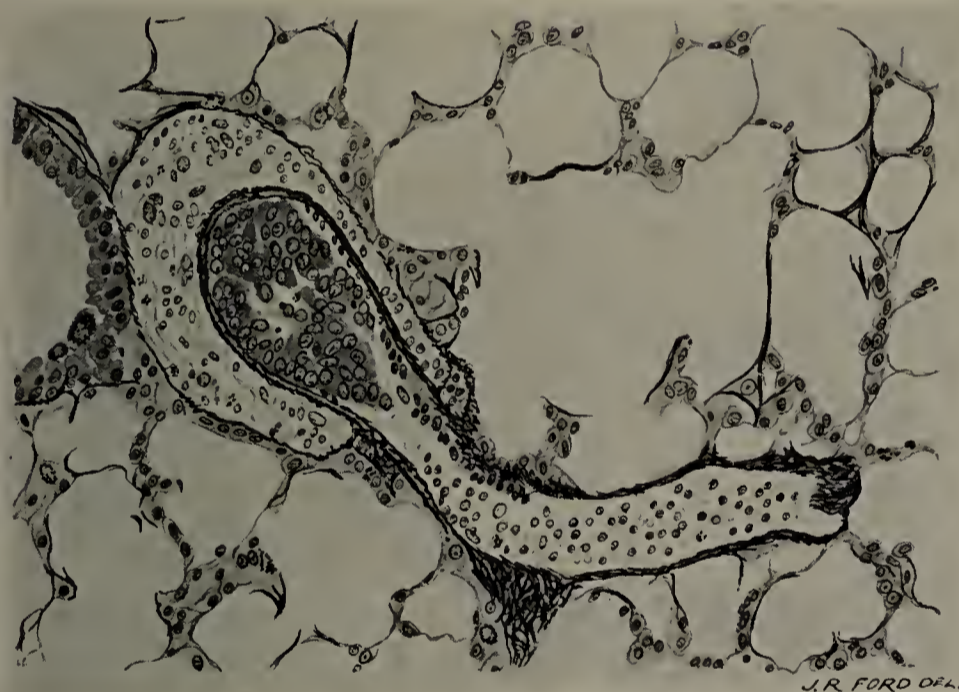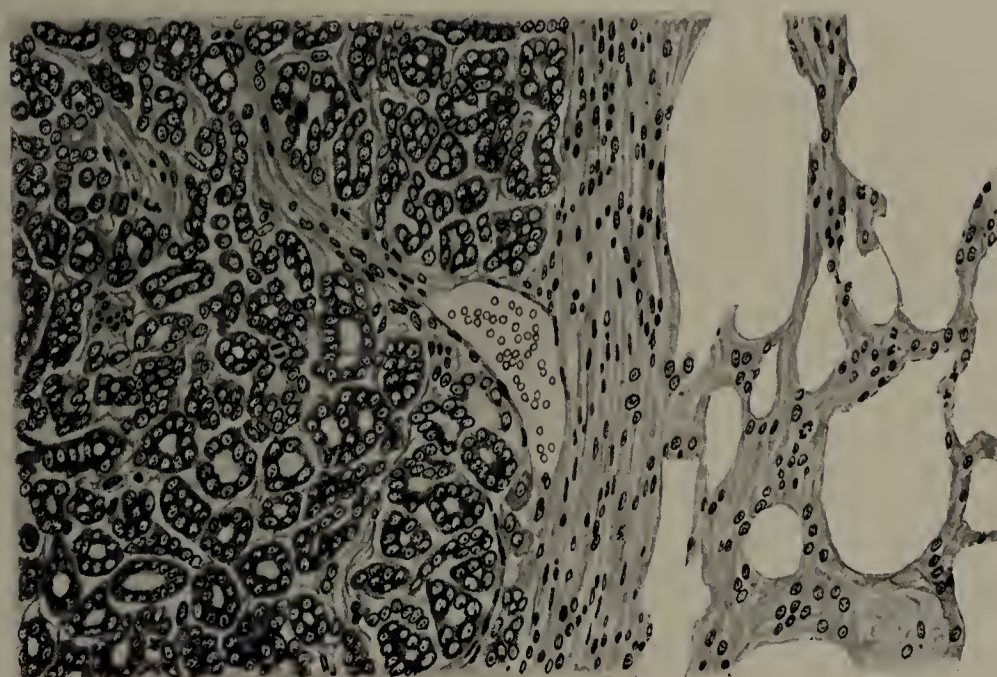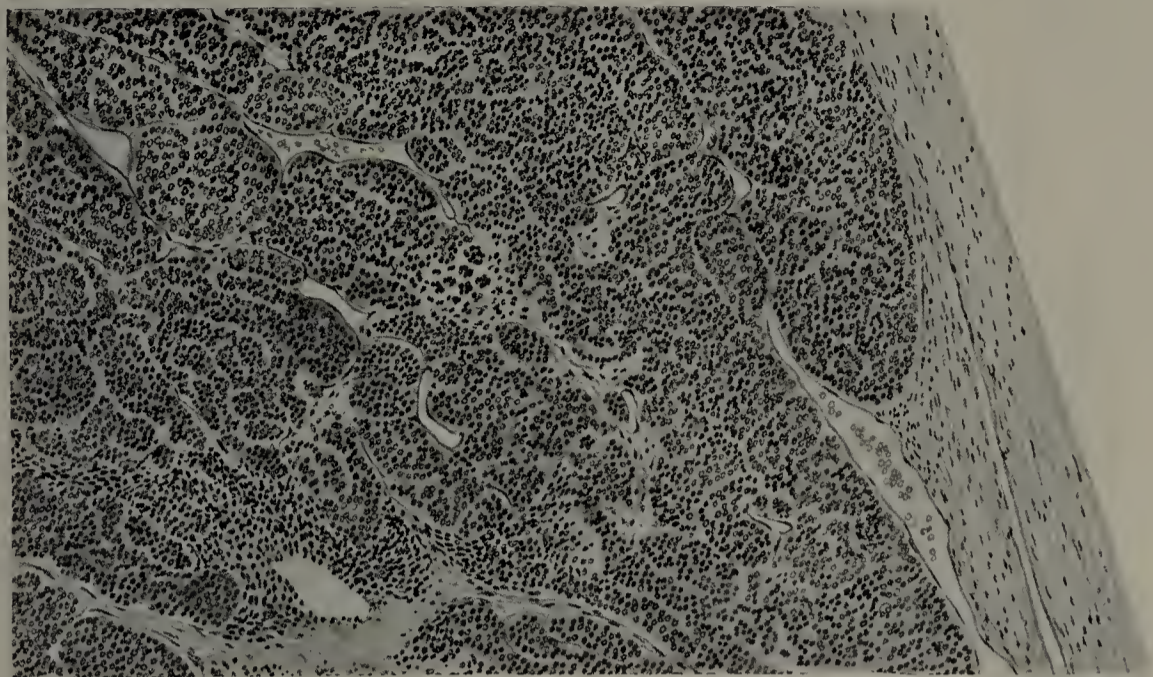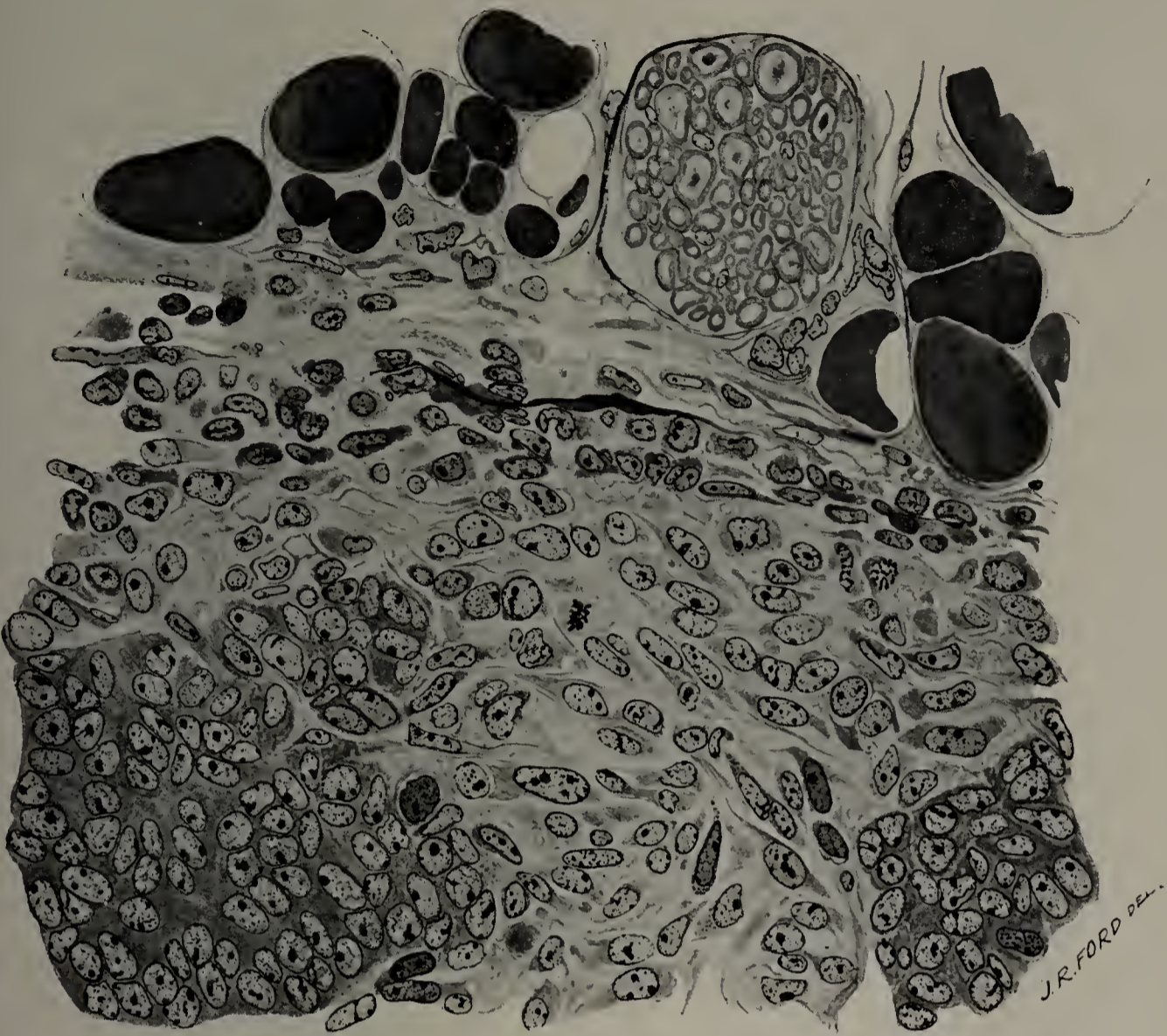
Fig. 1.

J.R. FORD DEL.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

PLATE XX

Fig. 6

Fig. 7.

Fig. 8.

Fig. 9.